Science Library

MAR 1 3 1947

Volume 37 *Number 3*

Phytopathology

AN INTERNATIONAL JOURNAL

March 1947

Official Organ of

The American Phytopathological Society

$6.25 per year, Canada $6.00 per year, United States and dependencies $6.50 per year, elsewhere

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter. Acceptance for mailing at the special rate of postage provided for in paragraph 4, Sec. 538, P. L. and R. Authorised May 12, 1921.

TABLE OF CONTENTS

The use of leaf tissue in graft-transmission of psorosis virus. JAMES M. WALLACE 149

A chemical study of the mycelium and sclerotia of Phymatotrichum omnivorum ..... DAVID R. ERGLE AND LESTER M. BLANK 153

Influence of certain environmental conditions on chlorotic streak of sugar cane ..... E. V. ABBOTT 162

Cercospora leaf spot of broad bean in China ..... T. F. YU 174

Helminthosporium rostratum on corn, sorghum, and Pearl millet. GEORGE Y. YOUNG, C. L. LEFEBVRE, AND A. G. JOHNSON 180

The effect of seedling diseases of castor beans on the subsequent plant development and yield ..... E. C. STEVENSON 184

Phytopathological Notes 189

Poria obliqua on dying beech ..... ROBERT A. ZABEL

The observed frequency of mature pycnidia of Septoria gladioli on gladiolus corms ..... DONALD P. LIMBER

Spotted wilt of broad bean ..... T. F. YU

A rough-bark disease of Pittosporum tobira. H. EARL THOMAS AND KENNETH F. BAKER

A fungicide for protecting lily bulbs from infection by Colletotrichum lilii ..... F. J. LEBEAU

The effects of carborundum in inoculating bean plants with bacteria ..... G. W. BOHN AND J. C. MALOIT

Efficacy of certain soil fumigants and fertilizers against crown rot in annual larkspur caused by Sclerotium rolfsii. W. D. MCCLELLAN

PHYTOPATHOLOGY

An International Journal
Official Organ of The American Phytopathological Society

EDITORS

HELEN HART, Editor-in-Chief, University Farm, St. Paul 1, Minnesota.
W. H. BURKHOLDER, Cornell University, Ithaca, New York.
A. J. RIKER, University of Wisconsin, Madison 6, Wisconsin.
H. A. RODENHISER, United States Horticultural Station, Beltsville, Maryland.

ASSOCIATE EDITORS

E. J. ANDERSON, Pineapple Research Institute, University of Hawaii, Honolulu, Hawaii.
G. M. ARMSTRONG, Clemson Agricultural College, Clemson, South Carolina.
L. M. BLACK, Brooklyn Botanic Garden, Brooklyn 25, New York.
DONALD E. BLISS, Citrus Experiment Station, Riverside, California.
H. LORAN BLOOD, Utah Agricultural Experiment Station, Logan, Utah.
F. L. DRAYTON, Central Experimental Farm, Ottawa, Canada.
A. L. HARRISON, Arizona Agricultural Experiment Station, Tempe, Arizona.
RAY R. HIRT, New York State College of Forestry, Syracuse 10, New York.
F. L. HOWARD, Rhode Island State College, Kingston, Rhode Island.
M. F. KERNKAMP, University Farm, St. Paul 1, Minnesota.
C. M. TUCKER, University of Missouri, Columbia, Missouri.
S. M. ZELLER, Oregon Agricultural College, Corvallis, Oregon.

BUSINESS MANAGEMENT

M. C. RICHARDS, Business Manager, University of New Hampshire, Durham, New Hampshire
L. J. ALEXANDER, Advertising Manager, Ohio Agricultural Experiment Station, Wooster, Ohio.

NOTICES

All communications regarding subscriptions and back numbers should be sent to M. C. RICHARDS, Business Manager, University of New Hampshire, Durham, New Hampshire.

Subscription price. $6.00 per year United States and dependencies; Canada, $6.25; other countries $6.50; single numbers of the current volume, 60 cents each. The Journal has been issued monthly beginning with Volume 8, January, 1918. Prior to that it was issued bimonthly.

Separate Numbers may be purchased from broken volumes, and stocks available are as follows: Vol. 1, No. 1, index; Vol. 2, Nos. 1, 5, index; Vol. 3, No. 5; Vol. 6, index; Vol. 8, Nos. 2, 3, 5, 6, 7, and 9 through 12; Vol. 9, Nos. 2 through 12, index; Vol. 10, Nos, 2 through 12. Separate numbers of volumes 1 through 10 are sold at $2.00 per copy, those of volumes 11 through 35 at $1.25 a copy. Complete, unbound volumes 11 through 35 are available at $10.00 a volume.

Thirty-Year Index (to Vols. 1–30, 1911–1940) bound. $6.25, United States and Canada. $7.50, elsewhere.

Advertisements. Rates may be secured from the Advertising Manager. No indorsement of any statement of claims made in advertisement is assumed by this journal or by The American Phytopathological Society.

Lost Numbers. Claims for copies (domestic subscriptions) lost in the mails must be received within 30 days from the date of issue (Jan. 15 and monthly thereafter); otherwise the usual charge of 60¢ per copy will be made for replacements. Deliveries overseas are not guaranteed and foreign mailings are made at the subscriber's risk.

Separates. No gratis copies supplied. A printed schedule of prices is submitted with the proof, and authors may secure separates by placing an order on the blanks provided when proof is returned.

Manuscript may be sent to the nearest member of the editorial board. Clearness, brevity, and conciseness are essential. In form and style, such as punctuation, spelling, and use of italic, manuscript should conform to the best usage in recent numbers of this journal. Manuscripts should be typed on one side of the paper, double spaced, and sent unfolded. Papers longer than four printed pages should include a summary. The name and address of the institution at which the work was done should be included at the end of the summary. Footnotes should be numbered consecutively with arabic numerals except in tables, where small letters should be used. Each table should have a heading stating clearly and concisely its contents. References to literature should be made as footnotes only when five or fewer citations are given. The simplified method of citing literature used in recent numbers of this journal should be followed. References in "Literature Cited" should be listed aphabetically and numbered consecutively. Authors are advised to read "The preparation of manuscripts for Phytopathology" by A. J. Riker, published in Phytopath. 36: 953–977. 1946.

Illustrations necessarily must be limited in number. Photographs, to reproduce satisfactorily, must be of best quality. Authors who desire unusual numbers of illustrations or special types of illustrations will be asked to bear part of the cost, i.e. in excess of $12.50, the approximate cost of 2 full-page cuts. All illustrations are reproduced on the same grade of paper and will be run as text figures designated by arabic numerals. Individual illustrations within a text figure should be designated by letters. Full-page figures should be arranged to conform to journal page size and shape ($4\frac{1}{2} \times 7$ inches) after making due allowance for reductions. Specific reference to each illustration must be made in the text. Each illustration must be accompanied by an adequate legend, the legend to be typed on separate paper and not on the illustration.

The responsibility for statements, whether of fact or opinion, rests entirely with the writers thereof.

The American Phytopathological Society

FOUNDED 1909. INCORPORATED 1915

The A. P. S. Council for 1947

President—A. J. Riker, University of Wisconsin, Madison 6, Wisconsin.
Vice-President—R. S. Kirby, Pennsylvania State College, State College, Pennsylvania.
Secretary—E. M. Johnson, Agricultural Experiment Station, Lexington 29, Kentucky.
Treasurer—M. C. Richards, University of New Hampshire, Durham, New Hampshire.
Editor-in-Chief—Helen Hart, University Farm, St. Paul 1, Minnesota.

Councilors:

S. G. Lehman, N. C. State College, Raleigh, North Carolina.
R. J. Haskell, Extension Service, U. S. Dept. Agr., Washington 25, D. C.
Thomas Sproston, Jr., University of Vermont, Burlington, Vermont.
James H. Jensen, North Carolina State College, Raleigh, North Carolina.
Max W. Gardner, University of California, Berkeley, California.
C. M. Tucker, University of Missouri, Columbia, Missouri.
J. H. Craigie, Central Experimental Farm, Ottawa, Canada.

Sustaining Associates:

B. F. Goodrich Chemical Company, Rose Building, Cleveland 15, Ohio.
Mallinckrodt Chemical Works, Second and Mallinckrodt Street, St. Louis 7, Missouri.
Givaudan-Delawanna, Inc., 330 West 42nd Street, New York 18, New York.
Rohm and Haas, Washington Square, Philadelphia 5, Pennsylvania.
E. I. du Pont de Nemours & Company, Wilmington 98, Delaware.
Freeport Sulphur Company, New Orleans 5, Louisiana.
Tennessee Corporation, 619–27 Grant Building, Atlanta 1, Georgia.
Flax Institute of the United States, Roanoke Building, Minneapolis, Minnesota.
Pillsbury Mills, Inc., Metropolitan Building, Minneapolis, Minnesota.
Rogers Bros. Seed Co., Inc., Idaho Falls, Idaho.
F. H. Woodruff and Sons, Milford, Connecticut.
Minnesota Valley Canning Co., Le Sueur, Minnesota.
Triarch Botanical Products, Ripon, Wisconsin.
Yoder Brothers, Inc., Barberton, Ohio.
Butler County Mushroom Farm, Inc., West Winfield, Pennsylvania.
Pabst Brewing Company, Milwaukee, Wisconsin.
Associated Seed Growers, Inc., New Haven 2, Connecticut.
Ferry-Morse Seed Co., Detroit, Michigan.

THE USE OF LEAF TISSUE IN GRAFT-TRANSMISSION OF PSOROSIS VIRUS[1]

JAMES M. WALLACE[2]

(Accepted for publication November 16, 1946)

INTRODUCTION

Inoculation of citrus with psorosis viruses by means of small bark-patch grafts was reported recently.[3] Further investigations have shown that similar grafts, using leaf instead of bark tissue, give equally satisfactory transmission. This proves to be an advantage in that it speeds up inoculation procedure and eliminates the difficulty sometimes encountered in cutting bark patches from specimens from which the bark does not "slip" easily.

Sreenivasaya[4] reported transmission of the virus of the spike disease of sandal when rectangular pieces of leaf tissue from diseased plants were placed under bark flaps of healthy plants. Inasmuch as spike-disease virus is not transmissible by means of juice inoculations, there was probably actual union of the tissue in such "grafts."

Cochran and Rue[5] have reported that the virus of peach mosaic has been transmitted by placing diseased leaf tissue under the bark of healthy peach trees.[6] They observed that the patches were alive 75 days after grafting.

INOCULATION METHODS

In the inoculation of citrus with the psorosis virus by means of leaf-tissue grafts, the procedure is much the same as that previously described by Wallace for bark-patch inoculation. Rectangular pieces are cut from leaves of diseased trees and placed under bark flaps on healthy trees. The bark flap is folded back in position over the inserted leaf piece and held in place by a strip of adhesive rubber tape. It has been found that tissue from very young, newly formed leaves, as well as from fully mature leaves, can be used for transmission of the psorosis virus. The leaf piece need not fit exactly the exposed wood under the bark flap but should be small enough to be completely covered by the flap, so as to provide more opportunity for tissue union.

[1] Paper No. 550, University of California Citrus Experiment Station, Riverside, California.

[2] Associate Plant Pathologist in the Experiment Station.

[3] Wallace, James M. Technique for hastening foliage symptoms of psorosis of citrus. Phytopath. 35: 535–541. 1945.

[4] Sreenivasaya, M. Contributions to the study of spike disease of sandal. Part XI. New methods of disease transmission and their significance. Indian Inst. Sci. Jour. 13 A (Pt. X): 113–117. 1930. (Original not seen by present writer; citation (*68*), p. 214 *in*: Smith, Kenneth M. Recent advances in the study of plant viruses. P. Blakiston's Son and Co., Inc., Philadelphia. 1934.)

[5] Cochran, L. C., and John L. Rue. Some host-tissue relationships of the peach mosaic virus. (Abstr.) Phytopath. 34: 934. 1944.

[6] In conversation, L. C. Cochran has informed the writer that, prior to the tests conducted by himself and Rue, mosaic infection resulted in peach trees inoculated by H. H. Thornberry by means of macerated leaf tissue placed under the bark; and that since peach mosaic is not transmissible by juice inoculations, the transmission obtained is assumed to have resulted from tissue union.

If the leaves used are fresh and clean, and if the bark of the inoculated tree "slips" easily, very few of the leaf sections fail to unite with the inoculated trees. Occasionally, however, the leaf sections dry or decay and transmission does not take place. In some instances the inoculated trees have not developed symptoms even though the leaf sections remained alive for a long time. To test the efficiency of this method of inoculation, 10 separate experiments were conducted with leaves of different ages and with different degrees of symptoms. The total number of trees included in the inoculation tests was 174, and of these, 160, or approximately 92 per cent, developed psorosis symptoms. This method is now being used almost exclusively by the writer in psorosis studies, and many inoculation series have resulted in 100 per cent infection.

Seedling trees used in transmission studies were usually 5 to 7 mm. in diameter near the base of the main stem. Any tree that is of sufficient trunk diameter to permit peeling of a small bark flap can be inoculated in this manner, but it is somewhat easier to make this type of graft if the trees are 5 mm. or more in diameter.

Microscopic examination of sections of some of the leaf-tissue grafts showed that an increase in the amount of cut surface of the leaf piece provided more tissue capable of developing callus and thus gave more opportunity for tissue connections to form between the leaf pieces (the inoculum) and the trees in which they were placed. The procedure followed thereafter accordingly included an additional longitudinal cut through the center of the leaf piece, from points near the opposite ends. If leaf pieces included the midrib, a thin layer of tissue was shaved from the raised portion of the midrib.

A razor blade is suitable for making the cuts in preparing the bark flap, and for cutting the leaf pieces. A budding knife or scalpel is used to pry out the bark at the crosscut so as to permit the bark flap to be peeled downward.

ANATOMICAL STUDIES OF LEAF-TISSUE GRAFTS

The fact that the psorosis virus was transmitted from the leaf tissue to the seedling trees was evidence that a union of the tissues of the respective parts took place. Microscopic examination revealed the manner in which the tissues became united, and showed that union occurred only along the cut edges of the leaf pieces. Whenever entire, uninjured leaves were placed under the bark, there was no union.

Figure 1, A, shows a portion of a leaf-tissue graft sectioned and photographed 20 days after being placed under the bark. Callous tissue had formed at the cut edge of the leaf piece and had united with that formed by the cambium of the tree. It is evident that callous tissue developed from the direction both of the bark and of the wood. Where the cuticle and epidermis of the inserted leaf piece were not broken, there was no tissue union. When sections were mounted in water, the leaf portion separated

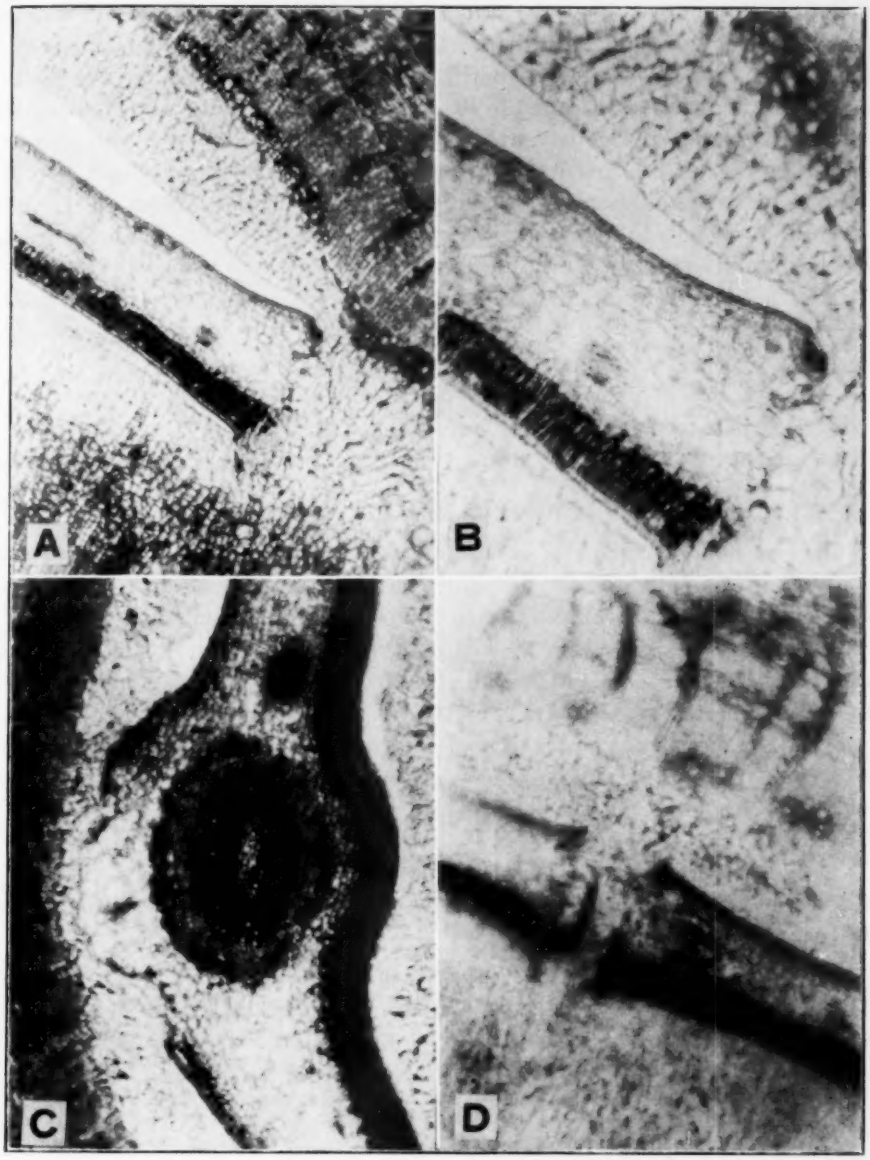

FIG. 1. A. Section of leaf-tissue graft 20 days after tissue was placed under bark flap. Note fusion of callous tissue at cut edge of leaf patch, and lack of connection where leaf epidermis was unbroken. ×72. B. Enlarged view of same section. ×144. C. Section through leaf-tissue graft in which midrib of leaf was included. Callus union resulted where the surface of the midrib was removed. ×72. D. Leaf-tissue graft in which additional cut was made through leaf piece. One month after grafting, callus had grown completely through the opening, and new xylem was again being produced on the bark side. ×72.

and floated free except at the attached edge. Figure 1, B, shows an enlarged view of the point of union of the respective parts.

Figure 1, C, shows a section through a leaf-tissue graft in which the midrib of the leaf was included in the leaf piece. A thin layer of tissue had been shaved from the under surface of the midrib. The unbroken surface of the leaf piece was placed adjacent to the xylem, with the bark flap in contact with the cut surface of the midrib. Fusion of tissues took place where the surface of the leaf piece was injured, but there was no tissue union on the opposite side, where the cuticle and epidermis were unbroken.

When a longitudinal cut was made through the leaf piece, callus formed by the seedling tree filled the opening and united with the cut edges of the leaf piece, as shown in figure 1, D.

Examination of some of the leaf pieces 30 months after they were placed under the bark showed them to be living and to have a normal green color, although they were then buried in 3 to 4 mm. of wood.

DISCUSSION AND SUMMARY

Inoculation by means of small, rectangular pieces of leaf tissue from psorosis-affected citrus trees, placed under bark flaps of healthy citrus trees, resulted usually in infection of 90 to 100 per cent of the inoculated trees.

Microscopic examination showed that callous tissue developed along the cut edges of the leaf piece and united with callus formed by cambial tissues of the inoculated tree. Fusion of tissues was rapid. The appearance of psorosis symptoms on the inoculated seedlings within 2 to 4 weeks, depending upon the rate of growth of new shoots, showed that the psorosis virus moved into them from the leaf pieces (the inoculum) within a few days after the grafts were made.

Numerous leaf pieces examined 30 months after being placed under the bark were still living and were of normal green color even though they were then buried in 3 to 4 mm. of wood.

Citrus may be especially adapted for the type of leaf-tissue graft described in this paper, but the successful transmission of viruses of sandal and peach by similar grafts suggests that this method may be applicable to a wide range of plants. It has not yet been determined whether virus transmission can be accomplished with this technique between plants that are not compatible when budded or grafted by the usual methods. If such should prove to be the case, studies of the host range of virus disease will be facilitated.

With plants in which this method of grafting can be used for virus transmission, inoculation procedure is simplified and inoculations can be made rapidly. The method may also prove of value in studies of rate of movement and distribution of viruses in plant tissues.

UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA.

A CHEMICAL STUDY OF THE MYCELIUM AND SCLEROTIA OF PHYMATOTRICHUM OMNIVORUM[1]

DAVID R. ERGLE AND LESTER M. BLANK

(Accepted for publication November 20, 1946)

Since 1888 (11) *Phymatotrichum omnivorum* (Shear) Duggar, a soil-inhabiting fungus, has been the subject of a vast amount of research. Much of the earlier work has been summarized by Rea (12) and Streets (14). Information on the chemical composition of the fungus appears to be limited to that by King (8), who found the conidia to contain 1.73 per cent organic nitrogen, and by Rogers (13), who reported, on the basis of microchemical tests, the presence of cellulose, protein, starch, and suberin in naturally occurring sclerotia recovered from field soils.

In the study reported in this paper *Phymatotrichum omnivorum* was cultured in three different soil types containing sorghum seed as the primary source of food, the variations in certain constituents of sclerotia during growth were determined, and these are considered in the light of their possible physiological significance. A similar study was made of mycelium grown in a nutrient solution. In the field this form of the organism, which brings about root infection of susceptible hosts, may be seen as a delicate weft of slender filaments and heavier strands, tan to buff colored, encompassing the roots.

Past investigations of the fungus relative to its occurrence and activity in certain soil areas of the Southwest have justified two conclusions that were considered in planning the work described in this study. (a) Sclerotia, which are compact tuber-like swellings of large mycelial strands, constitute one of the most important means by which the fungus persists in the soil. They are known (16) to have remained viable in the soil for at least 8 years, and have been found in large quantities to depths of 8 feet in Houston Black clay soil (13). (b) Soil type, composition, and reaction appear to be important factors determining the ability of the fungus to maintain itself and the resulting severity of the disease (5, 6, 18). The extent of infestation and severity of damage by the fungus is favored in heavy soils of neutral to moderately alkaline reaction and inhibited in the acid soils.

CULTURE AND PREPARATION OF PHYMATOTRICHUM OMNIVORUM FOR CHEMICAL ANALYSIS

Because of the importance attached to soil type in determining the distribution and activity of the fungus, three soils associated with varying degrees of natural infestation were used as substrates for obtaining suitable quantities of sclerotia for chemical study. They were as follows: (a) Houston Black clay, high-lime phase (14.44 per cent CaO), pH 8.1, obtained from

[1] Published with the approval of the Director of the Texas Agricultural Experiment Station as Technical Paper No. 969.

a field highly infested with *Phymatotrichum omnivorum* near Temple, Texas. (b) Houston Black clay, low-lime phase (1.55 per cent CaO), pH 7.9, obtained near Moulton, Texas, from a field in which the degree of infestation appears to be less than in (a). (c) Wilson clay, noncalcareous, pH 4.4, obtained near Greenville, Texas, in a field in which there was little infestation.

Sclerotia were obtained by the soil-culture method of Dunlap (2) whereby the fungus was cultured in 100 g. soil plus 35 ml. tap water, and 5 g. sorghum seed spread over the surface. The mixture, contained in a 250-ml. Erlenmeyer flask, was sterilized by autoclaving. Inoculation was by means of a small disc of nutrient agar containing an established culture of the fungus isolated from a naturally infected cotton plant. Using the soils described above, three series of 65 flasks each were thus prepared and incubated at 28° to 30° C. Seventeen days after inoculation, the sclerotia formed by the fungus were separated from the soil in 20 flasks of each soil type by washing with water and screening. After removal of the surface water by blotting, the sclerotia were weighed as a composite sample for each soil type, dried in a forced draft oven at 75° C., and then reweighed to obtain a dry weight of the sclerotia. Preparation for immediate analysis consisted of grinding in a small Wiley mill to pass an 80-mesh screen and redrying for weighing of analytical samples. Similar harvests of sclerotia from 15 flasks in each series were made 33, 49, and 65 days after inoculation, representing successive stages in sclerotial growth and development. Preliminary experiments had demonstrated that the peak in sclerotial growth was reached in approximately 65 days of incubation under the conditions described.

While sclerotia grew best in soil cultures, the mycelium could not be separated in quantity free of soil particles. So mycelium for chemical analysis was grown on 50 ml. of nutrient (15) solution in 250-ml. Erlenmeyer flasks. Inoculation and incubation were like those for the soil cultures. When fungal growth was near its maximum after 16 days, the mats from 15 flasks were harvested, washed free of nutrient solution, dried for 3 hours, and prepared for analysis as described for sclerotia. A second harvest was made after 22 days from 15 more flasks. During these last 7 days autolysis began. The mean dry weight of fungal mat per flask at the first harvest was 867 mg. and at the second harvest was 833 mg.

ANALYTICAL PROCEDURE

Ash, crude fat, and organic nitrogen (nitrates were absent) were determined by A.O.A.C. official methods (1). Crude protein was calculated by multiplying nitrogen by the usual conversion factor, 6.25.

The sugars were Soxhlet-extracted with 80 per cent alcohol and determined by the method of Wildman and Hansen (19). Nonreducing sugars were determined after hydrolysis with 4 per cent hydrochloric acid (by weight) for 1.25 hours at 15 lb. pressure. The methods of analysis as out-

lined by Loomis and Shull (10) formed the basis for the following determinations in the order given: (a) Glucosans and pectin were determined on separate aliquots of a hot-water extract of the residue from the extraction of sugars. Pectin was precipitated as the calcium salt according to the procedure of Emmet and Carré (4). (b) The residue from (a) was hydrolyzed with 2.5 per cent hydrochloric acid for 1 hour at 15 lb. pressure and separate aliquots of the hydrolysate used for the determination of hemicellulose and pentosans. (c) The washed and dried residue from (b) was treated with 72 per cent sulphuric acid for 24 hours at 8° C. followed by a 3 per cent sulphuric acid hydrolysis at 100° C. for 2 hours. The reducing power of the hydrolysate, calculated as anhydrous dextrose, was used as an index of the cellulose content. Inasmuch as tests for lignified tissue in mycelium and sclerotia were negative whereas the presence of suberin in sclerotia was confirmed (13), the residue from the cellulose determination, after correcting for its ash content, was reported as suberin.

The presence of suberin was confirmed by the formation of a yellow to brown color when sections of fresh tissue were treated with chloro-zinc iodide (7). Glycogen was identified by the opalescence of the glucosan extract, by a reddish brown color with iodine that was intensified by sodium chloride and by the conversion to glucose upon acid hydrolysis (9).

RESULTS

It is believed that sclerotia formation was limited for the most part to the initial 17-day incubation period and that the succeeding periods represent their continued growth and development. Such an assumption is supported by two types of experimental evidence. First, sclerotia formed in the 17-day-old cultures were invariably lighter in color and smaller than those in older cultures. Second, the nutrient reserves of sclerotia increased consistently with length of incubation period, thus indicating that they were older physiologically.

Sclerotia

Analytical data on the changes in chemical composition of sclerotia with age of culture in the three soils are recorded in table 1 as percentage of fresh weight. The production and cumulated growth of sclerotia in the three soils over the 65-day incubation period is shown in figure 1, A. Each point on the curves is the mean dry weight of sclerotia per culture computed from not less than 15 replicates. As shown by these curves the efficiency of the fungus in sclerotia production was in the following order: Houston Black clay—high lime, Houston Black clay—low lime, and Wilson clay. These results confirm similar observations reported by Dunlap (2).

As illustrated in figure 1, B, growth was accompanied by a marked decrease and ultimate disappearance of reducing sugars from the sclerotia. The concentration of reducing sugars in sclerotia varied inversely with rate of growth in the three soils whereas the rate at which these sugars disappeared tended to be directly related to growth. This is interpreted as

indicating an effect of soil type on the ability of sclerotia to utilize the sugars made available by enzymatic hydrolysis of the sorghum seed. Apparently the sclerotia in the Wilson soil were getting adequate amounts of sugars, as indicated by a higher content, but were unable to convert them into the various products of synthesis as fast as sclerotia growing in the two Houston soils.

TABLE 1.—*Chemical composition of Phymatotrichum sclerotia at four stages of growth in three different soils. Results given as percentage of fresh weight*

Soil type and constituents	Age of culture—days			
	17	33	49	65
Houston Black clay (High lime)				
Moisture	74.24	60.76	57.53	60.34
Ash	1.80	1.48	1.36	1.30
Fat	0.55	0.80	0.89	0.88
Protein	4.70	6.13	7.73	7.84
Reducing sugars	0.26	0.04	0.00	0.00
Nonreducing sugars	.....	0.56	0.48	0.68
Glucosans	1.26	3.86	4.37	6.07
Hemicellulose	8.37	13.34	14.64	14.84
Pentosans	.....	0.16	0.17	0.15
Pectin	.....	0.80	1.15	0.83
Cellulose	0.28	0.33	0.39	0.39
Suberin	0.33	0.82	0.73	0.54
Houston Black clay (Low lime)				
Moisture	75.20	62.24	59.73	57.03
Ash	1.67	2.07	1.79	1.38
Fat	0.62	0.89	0.96	1.13
Protein	4.26	5.85	6.79	7.55
Reducing sugars	0.31	0.16	0.03	0.00
Nonreducing sugars	.....	0.63	0.63	0.84
Glucosans	1.63	3.11	4.85	6.28
Hemicellulose	8.57	14.20	14.24	16.16
Pentosans	.....	0.11	0.15	0.16
Pectin	.....	0.65	1.24	0.67
Cellulose	0.26	0.31	0.46	0.36
Suberin	0.36	0.53	0.60	0.54
Wilson clay (Noncalcareous)				
Moisture	75.76	62.46	60.45	57.34
Ash	2.47	2.80	2.88	1.74
Fat	0.48	0.74	1.05	1.20
Protein	4.53	5.77	6.27	7.52
Reducing sugars	0.44	0.33	0.06	0.00
Nonreducing sugars	.....	0.53	1.07	0.99
Glucosans	0.68	3.05	3.35	4.00
Hemicellulose	7.47	15.73	12.92	18.30
Pentosans	.....	0.21	0.15	0.20
Pectin	.....	0.56	0.66	0.80
Cellulose	0.26	0.32	0.34	0.41
Suberin	0.40	0.53	0.72	0.66

Concurrently with growth and development, the sclerotia, irrespective of soil type, increased consistently in concentrations of fat, protein, glucosan, and hemicellulose. The capacity of sclerotia to accumulate protein and glucosans (Fig. 1, C and D) was apparently influenced moderately by the kind of soil used as culture medium.

The concentrations of nonreducing sugars, pentosans, pectin, cellulose, and suberin in sclerotia remained relatively small throughout the growth

period and were not affected by the nature of the substrate. Excepting pentosans these constituents did, however, tend to increase in concentrations as the sclerotia approached maturity.

The moisture content of sclerotia decreased with age without any differences attributable to soil type. The ash content varied inconsistently with respect to growth but was always present in higher concentrations in sclerotia from the Wilson soil.

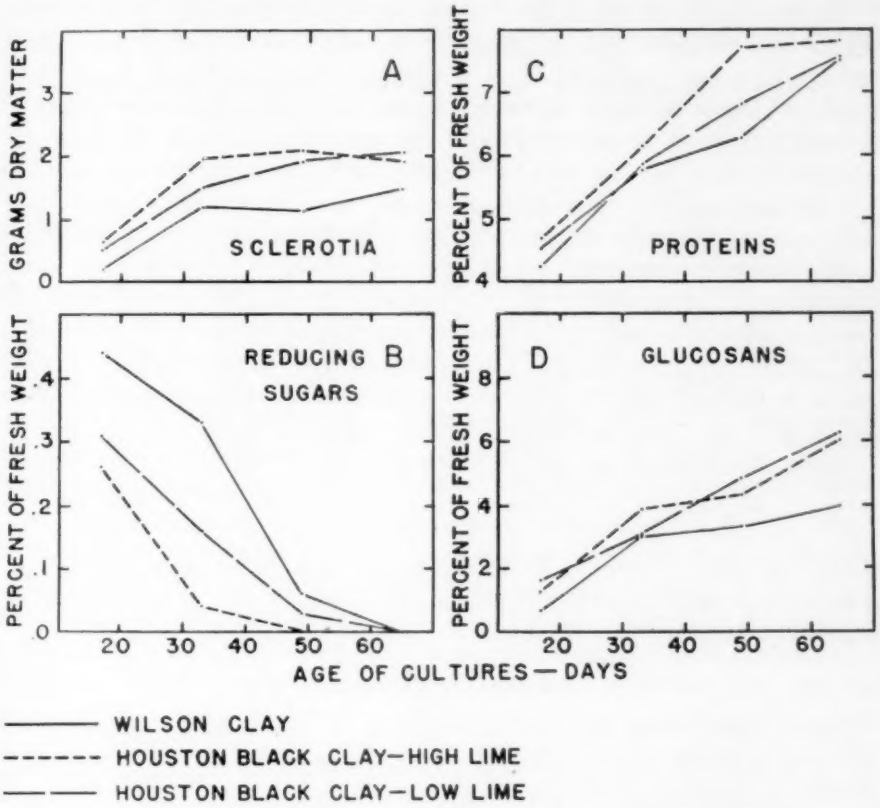

FIG. 1. Graphs showing the influence of soil type on (A) growth of sclerotia; (B) rate of disappearance of reducing sugars; and accumulation during growth of (C) proteins and (D) glucosans.

Mycelium

The data comparing the chemical composition of mycelium and sclerotia at two stages of comparable physiological age are recorded in table 2 as percentage of dry weight. The use of the moisture-free basis was advisable due to the absorption of water by the mycelial mats during washing, thereby precluding a representative fresh weight.

As grown, the mycelium reached a maximum weight in 16 days and, as a result of some autolysis, was declining by the 22nd day. This is about half of the growth period of sclerotia in the soil culture. The mycelium at

all dates of measurement was higher than the sclerotia in concentrations of ash, fat, protein, reducing sugars, pectin, cellulose, and suberin. Sclerotia, on the other hand, were definitely higher in concentrations of nonreducing sugars, glucosans, and hemicellulose. At the peak of growth, mycelium and sclerotia had accumulated total carbohydrates (sugars + glucosans + hemicellulose) in concentrations of 23.16 and 45.92 per cent of their dry weights, respectively. With reference to the types of carbohydrates accumulated, the mycelium was richer in sugars whereas sclerotia were more abundantly supplied with polysaccharides. This is a more or less expected

TABLE 2.—*Comparison of chemical composition of mycelium and sclerotia of Phymatotrichum at two stages of growth. Results given as percentage of dry weight*

Constituents	Mycelium			Sclerotia		
	Age of culture—days		Means	Age of culture—days		Means
	16	22		49	65	
Ash	6.12	6.35	6.24	3.20	3.29	3.25
Fat	4.03	4.95	4.49	2.10	2.23	2.17
Protein	23.75	24.13	23.94	18.19	19.77	18.98
Reducing sugars	4.74	4.34	4.54	0.00	0.00	0.00
Nonreducing sugars	0.00	0.00	0.00	1.14	1.72	1.43
Glucosans	1.48	0.93	1.21	10.30	15.30	12.80
Hemicellulose	16.94	20.37	18.66	34.48	37.41	35.95
Pentosans	0.45	0.47	0.46	0.41	0.38	0.40
Pectin	6.36	3.10	4.73	2.71	2.09	2.40
Cellulose	2.46	3.17	2.82	0.91	0.99	0.95
Suberin	2.69	2.86	2.78	1.73	1.36	1.55

result since the mycelium may be considered to be more active physiologically and of more temporary duration than sclerotia, and as such might be expected to accumulate carbohydrates in more labile forms.

Other Constituents

Some information was obtained relative to the chemical character of the carbohydrates involved in the synthetic processes of the two fungal forms. A nonreducing sugar, hydrolyzed to glucose with difficulty, was present in sclerotia but not in the mycelium. Although the identity of this compound was not definitely established, its behavior on acid hydrolysis is suggestive of trehalose, a disaccharide of fairly wide distribution among fungi. Upon strong acid hydrolysis trehalose yields two molecules of glucose. Neither starch nor sucrose was present in either of the two stages of the fungus. Thus the presence of starch in sclerotia, as reported by Rogers (13), was not confirmed. Hot water extracts of both mycelium and sclerotia contained a polysaccharide identified as glycogen that was included in the glucosan determination. Glycogen is a common reserve carbohydrate in other fungi such as mushrooms and yeast. The hemicellulose fraction in both types of tissue was low in pentosans and upon acid hydrolysis yielded glucose as the principal sugar. There was some evidence that glycogen

was present also in the hemicellulose fraction. These findings indicate that the synthesis of polysaccharides in this fungus was limited for the most part to the utilization of glucose as polymer units.

DISCUSSION

The results of this study aid materially in explaining from a nutritional standpoint why the sclerotial form of *Phymatotrichum omnivorum* is able to play an important rôle in the perpetuation of the organism in field soils. The demonstrated ability of sclerotia during growth to accumulate carbohydrates, fat, and protein in relatively high concentrations suggests that their physiological function is primarily that of a storage organ. Such a concept accords with the known behavior of this form of the fungus as it occurs naturally in soils infested with *Phymatotrichum omnivorum*. In such an environment, sclerotia may remain dormant for long periods but dormancy may be broken by the advent of favorable moisture and temperature. The breaking of dormancy results in the formation of young hyphae which are capable of attacking the roots of susceptible hosts. It is obvious that the initial phase of hyphal production and expansion is at the expense of stored reserves, and that the amount of growth, independent of an auxiliary source of organic nutrients, is conditioned by the amount of stored nutrients. Therefore, the fungus with an abundant supply of food stored in sclerotia could be expected to survive for a time in soils kept free of susceptible plants.

The data comparing the chemical composition of the two fungal forms indicate that fat, protein, and carbohydrates can be expected to accumulate during growth in mycelium as well as in sclerotia. This similarity is not surprising in view of the fact that sclerotia are formed from mycelial strands. However, in view of the lower percentage of carbohydrates in the mycelium, it seems likely that the independent existence of the fungus in this stage would encounter a critical shortage of carbohydrates sooner than the sclerotial stage. Thus it is apparent that the mycelium is not so well adapted as sclerotia to prolonged survival under critical conditions.

The occurrence of suberin in mycelium and sclerotia may have a substantial significance from the standpoint of moisture retention. It has been shown elsewhere (3) that *Phymatotrichum omnivorum* is capable of making considerable growth from moist substrates out into soils of moisture content within the wilting range.

SUMMARY

Phymatotrichum omnivorum, a soil-inhabiting fungus, was cultured in (a) Houston Black clay, high-lime phase, (b) Houston Black clay, low-lime phase, and (c) Wilson clay soil, and the chemical composition of the sclerotia was determined at four stages of growth. Growth of sclerotia was accompanied by a disappearance of reducing sugars at a rate commensurate with growth and an accumulation of fat, protein, glucosans, and hemicellu-

lose. Corresponding with a higher rate of growth, glucosans and protein tended to accumulate in higher concentrations in sclerotia produced in the two Houston soils than in the Wilson soil. Nonreducing sugars, pectin, pentosans, cellulose, and suberin were present in sclerotia in relatively small amounts which, with the exception of pentosans, tended to increase with the approach of maturity but were not affected by soil type.

The mycelium of *Phymatotrichum omnivorum*, cultured in a suitable nutrient solution, was found to contain fat, protein, and reducing sugars in higher concentrations than sclerotia but manifested a substantially lower capacity than the latter for storing total carbohydrates (sugars + glucosans + hemicellulose). The sclerotial stage was richer in nonreducing sugars, glucosans, and hemicellulose. Ash, pectin, pentosans, cellulose, and suberin were also present in mycelium in larger concentrations than in sclerotia.

Starch and sucrose were absent in both fungal forms. A disaccharide, believed to be trehalose, was present in sclerotia but absent in the mycelium. Glycogen was identified in both forms of the fungus. Excepting the occurrence of small amounts of pentosans, the polysaccharides in both stages of the fungus appeared to be chiefly polymers of glucose.

BUREAU OF PLANT INDUSTRY, SOILS, AND
AGRICULTURAL ENGINEERING
AND
TEXAS AGRICULTURAL EXPERIMENT STATION,
COLLEGE STATION, TEXAS.

LITERATURE CITED

1. ASSOC. OFFIC. AGR. CHEM. Official and tentative methods of analysis. 4th ed. 1935.
2. DUNLAP, A. A. A convenient soil-culture method for obtaining sclerotia of the cotton root rot fungus. Amer. Jour. Bot. 28: 945–947. 1941.
3. EATON, F. M., E. W. LYLE, and D. R. ERGLE. Relations between carbohydrate accumulation and resistance of cotton plants to Phymatotrichum root rot in dry summers. In press.
4. EMMET, A. M., and M. H. CARRÉ. A modification of the calcium pectate method for the estimation of pectin. Biochem. Jour. 20: 6–12. 1926.
5. FRAPS, G. S. Relation of the occurrence of cotton root rot to the chemical composition of soils. Texas Agr. Exp. Sta. Bull. 522. 1936.
6. GILBERT, W. W. Cotton diseases and their control. U. S. Dept. Agr. Farmers' Bull. 1187: 27–31. 1921.
7. JOHANSEN, DONALD ALEXANDER. Plant Microtechnique. 1st ed. 523 pp. 1940.
8. KING, C. J. Habits of the cotton rootrot fungus. Jour. Agr. Res. [U.S.] 26: 405–418. 1923.
9. KOCH, FREDERICK C. Practical methods in Biochemistry. 1934.
10. LOOMIS, WALTER E., and CHARLES A. SHULL. Methods in Plant Physiology. 1st ed. 472 pp. 1937.
11. PAMMEL, L. H. Root rot of cotton or "cotton blight." Texas Agr. Exp. Sta. Bull. 4. 1888.
12. REA, H. E. The control of cotton root-rot in the Blackland region of Texas. Texas Agr. Exp. Sta. Bull. 573. 1939.
13. ROGERS, C. H. Cotton root rot studies with special reference to sclerotia, cover crops, rotation, tillage, seeding rates, soil fungicides, and effects on seed quality. Texas Agr. Exp. Sta. Bull. 614. 1942.
14. STREETS, R. B. Phymatotrichum (cotton or Texas) root rot in Arizona. Ariz. Agr. Exp. Sta. Tech. Bull. 71. 1937.

15. TALLEY, PAUL J., and LESTER M. BLANK. A critical study of the nutritional requirements of *Phymatotrichum omnivorum*. Plant Physiol. **16**: 1–18. 1941.
16. TAUBENHAUS, J. J. Laboratory studies on longevity of sclerotia. Texas Agr. Exp. Sta. Ann. Rept. 49. 1936.
17. ————————, W. N. EZEKIEL, and J. F. FUDGE. Relation of soil acidity to cotton root rot. Texas Agr. Exp. Sta. Bull. 545. 1937.
18. ————————, ————————, and D. T. KILLOUGH. Relation of cotton root rot and Fusarium wilt to the acidity and alkalinity of the soil. Texas Agr. Exp. Sta. Bull. 389. 1928.
19. WILDMAN, SAM G., and ELMER HANSEN. A semi-micro method for the determination of reducing sugars. Plant Physiol. **15**: 719–725. 1940.

INFLUENCE OF CERTAIN ENVIRONMENTAL CONDITIONS ON CHLOROTIC STREAK OF SUGAR CANE

E. V. Abbott[1]

(Accepted for publication November 21, 1946)

Wherever chlorotic streak, a virus disease of sugar cane, has been studied it has been noted that incidence and severity of the disease may be influenced by the conditions under which the sugar-cane plant is growing. It has been observed to be particularly severe where drainage is poor, and there have been indications that its severity is enhanced by nutritional deficiencies. However, there has been little experimental evidence to support the field observations. In this paper the results of experiments on the effect of soil moisture and texture and of certain fertilizers on the disease are reported.

SOIL MOISTURE

The greater prevalence of chlorotic streak in low, poorly drained areas as compared with well drained ones has been observed in Queensland (3, 4, 5), Hawaii (9), and Louisiana (1). One instance of the association of the disease with high soil moisture in Louisiana, apparently independently of other soil differences, may be cited as a typical example.

In June, 1940, infection counts were made in a commercial field of the variety C.P. 29/320 plant cane growing on "mixed" soil[2] in Terrebonne Parish, the rear portion of which was low and poorly drained in comparison with the front part. The area of poor drainage lay roughly at right angles to the direction of the rows, so that it was possible to make observations in poorly and well-drained portions of individual rows. The percentage of diseased plants in the low areas varied from 2 to 4 times that in the well-drained portions of the same rows, and in every row this unequal distribution of diseased plants occurred, with a rather sharp line of demarcation corresponding with the differences in drainage. This could scarcely be explained on the basis of unequal distribution of infected seed cuttings in different parts of the rows, since both the front and rear portions had been planted from the same wagon. Evidently, some effect of poor drainage had either resulted in a greater development of leaf symptoms, or in less recovery from the disease during germination of the buds and subsequent growth of the plants.

Experimental evidence has been obtained to support this and other similar field observations. In the fall of 1940, composite samples of chlorotic-

[1] Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

The author is indebted to R. L. Tippett and D. C. Bain for assistance in conducting some of the experiments.

[2] The so-called "mixed" soils in the Louisiana sugar-cane belt have not been differentiated as to soil type. They are intermediate between the predominantly sandy and predominantly clay loam soils, and comprise varying proportions of the components of each.

streak-infected stalks of 2 varieties, C.P. 807 and C.P. 29/103, were divided into 2 lots each, 1 for planting in a poorly drained end of a field of mixed soil and the other in a well-drained portion of the same field. There were 3 replications of 1/100-acre plots of each variety in each area, and in each plot 300 sound buds were planted. In 1941, when stands were established and chlorotic streak symptoms had appeared in the young plants, the percentages of diseased plants were determined. The number of plants of C.P. 807 with chlorotic streak symptoms was 54 per cent greater in the poorly drained than in the well-drained area, and in the C.P. 29/103 it was 78 per cent greater.

Since field observations had indicated that secondary spread of the disease was greater in areas of poor drainage, experiments were conducted to obtain more definite evidence.

In October, 1940, mature cuttings from apparently healthy plants of the variety C.P. 29/320 were treated with hot water at 52° C. for 20 minutes, which eliminates the disease from infected stalks, and divided into 2 lots for planting, respectively, in poorly and in well-drained portions of a field of "mixed" soil near Raceland, Louisiana. The field was 580 feet long. In the front 200 feet drainage was good, whereas in the rear 200 feet it was such that the soil remained wet for long periods following a rain, and water often stood for several days between the rows of cane, which in Louisiana are elevated about 12 to 18 inches. Frequent soil-moisture determinations showed that throughout the season the moisture content was consistently higher in the poorly drained area.

Four replications of 3-row plots, 1/100 acre in size, were planted in each of the 2 areas, using 300 sound buds per plot. Each plot was buffered by a row of chlorotic-streak-diseased cane on the sides, and by a similar 6-foot strip on each end. Thus, exposure to secondary spread of the disease by aerial insects should have been uniform in both areas.

In June, 1941, counts of primary shoots were made in all of the plots. At monthly intervals thereafter during the growing season as plant cane, and as first ratoon in 1942, the stools developing symptoms of chlorotic streak were marked with permanent wooden stakes. At the end of the ratoon year the percentage of infected stools was determined.

In October, 1941, a similar experiment was planted in a field in the same area and in which drainage conditions were similar. Eight varieties were included, with 9 replications of single-row plots 25 feet long in each of the areas of good and poor drainage. The seed cuttings were treated with hot water, the plots were buffered with chlorotic-streak-diseased cane, and notes were taken as described for the previous experiment. This experiment was continued through the crop seasons of 1942 and 1943. The results of the two experiments are in table 1.

Infection in the areas of poor drainage was significantly higher at the 5 per cent level for 7 of the 8 varieties included in the tests. Spread of the disease was greater in the area of poor drainage in the resistant varieties,

C.P. 33/372 and C.P. 34/21, as well as in the susceptible ones, although for the former variety the difference was not significant.

The influence of soil moisture on the development of chlorotic streak was also studied in the greenhouse, where variables other than moisture could be better controlled.

Greenhouse benches, $72 \times 38 \times 8\frac{1}{2}$ inches, were filled with fresh field soil, 2 with Sharkey silty clay and 2 with Yazoo very fine sandy loam. Composite samples of single-bud cuttings from chlorotic-streak-infected stalks of C.P. 29/320 were prepared by placing alternate buds successively in 4 lots as they were cut. After incubation at 35° C. for 36 hours, which caused the buds to swell, 50 were planted in each of the benches in groups of 10 on December 11, 1941. Soil moisture was kept at or near optimum in all of the benches

TABLE 1.—*Effect of drainage on secondary spread of chlorotic streak in 8 varieties of sugar cane*

Varieties used in each experiment	Plants infected with chlorotic streak				
	Good drainage	Poor drainage	Difference	Diff. Req'd. for P. = 0.05	Diff. Req'd. for P. = 0.01
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Experiment 1					
C.P. 29/320	16.4	29.8	13.4	2.7	9.0
Experiment 2					
C.P. 29/320	19.9	37.5	17.6	8.2	16.7
C.P. 32/206	42.8	55.4	12.6	9.1	18.7
C.P. 33/372	6.5	7.8	1.3	2.3	4.8
C.P. 34/21	4.1	7.4	3.3	2.2	4.6
C.P. 34/86	24.5	37.7	13.2	8.9	18.3
C.P. 34/92	22.5	30.9	8.4	4.9	10.2
C.P. 34/120	30.2	36.2	6.0	3.3	6.9
C.P. 34/139	35.6	58.3	22.7	8.0	16.4

until December 31, by which time the plants had become established. One bench of each soil was then flooded, and watered daily thereafter sufficiently to maintain the moisture level at within an inch of the surface of the soil. The soil in the other 2 benches was maintained at apparently optimum moisture content throughout the experiment.

When the experiment was terminated on March 9, 1942, 29 per cent of the plants in the sandy soil with high moisture had developed chlorotic streak symptoms, compared with 20 per cent in that with normal moisture; and in the heavy soil, 33 per cent developed symptoms with high moisture compared with 17 per cent with normal moisture. The differences were significant for both soils, confirming field evidence that high soil moisture is an important factor in favoring the development of chlorotic streak.

The foregoing experiments provide definite evidence not only of greater incidence of the disease among plants arising from infected seed when planted in poorly drained as compared with well-drained soils, but also of more rapid secondary spread, resulting apparently, from the greater susceptibility of plants growing under such conditions. It is possible that, as

suggested by Bell (4), the insect vectors of the disease are more active in the poorly drained areas. This is improbable, in the case of the one known insect vector *Draeculacephala portola* (2), which does not appear to be so limited in its movements as to confine itself to such relatively small areas in a field, but it is possible that the disease may be transmitted by some unknown soil-inhabiting vector that is more active in poorly drained areas. However, there are no data to support this, and the writer's efforts to transmit the disease through the soil have been unsuccessful. No infection of healthy plants occurred when these were grown for 9 to 10 months in alternate position with chlorotic-streak-infected ones in greenhouse benches of nonsteamed clay soil, the moisture content of which was maintained at a high level; nor when healthy plants were grown for 2 years in the greenhouse in cans of nonsteamed soil that had been collected from around the roots of diseased plants in the field.

In the field, extreme deficiency of soil moisture emphasizes the leaf scorching and streaking of diseased plants, so that during a drought the individual infected plants or areas of high infection stand out in sharp contrast to adjacent healthy ones. However, no evidence has been obtained to indicate that moisture deficiency results in higher incidence of the disease. On the contrary, in the field studies of secondary spread, more new cases appeared among originally healthy plants during the rainy summer months than during dry periods. It is realized, of course, that factors other than soil moisture, such as age and rate of growth of the plants, and seasonal activity of insect vectors, may be involved in the more rapid spread in summer.

SOIL TEXTURE

In Louisiana, poor drainage is associated more commonly with silty clay and clay soils, such as those of the Sharkey series (the so-called "black" lands), than with the lighter-textured sandy soils, such as those of the Yazoo series. The incidence of chlorotic streak has generally been found to be greater in sugar cane growing on the former as compared with the latter soils, and while it seemed probable that this was primarily because of the generally poorer drainage of the heavy soils, there remained the possibility that soil differences other than drainage might also have an influence on the disease. In the minds of many growers, at least, prevalence of the disease has come to be associated with the heavier soil types.

In order to determine whether soils of different texture held at or near their optimum moisture content would have any differential effect on the development of chlorotic streak, experiments were conducted in the greenhouse with sandy loam, and clay loam soil types and with muck soil.

Four composite samples of 150 single-bud cuttings from chlorotic-streak-infected stalks of the variety C.P. 29/320 were prepared as described previously for the greenhouse experiment. They were planted in 4-inch clay pots of soil, 2 lots of each in Yazoo very fine sandy loam and 2 in Sharkey

silty clay loam. One lot of each soil was germinated at 35°–36° C. and the other at 22°–23° C. As the shoots emerged from the soil, the pots were removed to benches in the greenhouse where the minimum air temperature was maintained at 24° C. The date of germination and of the first appearance of chlorotic streak symptoms was recorded for each plant. The experiment was begun in October, 1940, and terminated in August, 1941.

The two germination temperatures were used to determine whether greater recovery might occur in plants that germinated rapidly (at 35°–36° C., which is near the optimum for sugar cane), as compared with those germinating slowly. It seemed possible that the greater incidence of chlorotic streak in poorly drained areas might be at least partially the result of the slower rate of germination of plants in those areas. In the case of shoots arising from infected seed cuttings, less recovery might conceivably occur because of the slower rate of development of the plants during the cool winter and spring months that follow fall planting of sugar cane in Louisiana, as a result of which the virus would invade plants developing from infected buds to a greater extent than it would in those in well-drained areas where cane germinated and grew more rapidly. In other words, rapidly growing plants might grow away from the virus. Price (11) found this to be true in tobacco plants infected with ring spot.

In a similar experiment, plants of C.P. 29/320 were grown in 6-inch clay pots of the 2 soils, with germination at the greenhouse temperature (minimum 24° C.). The 64 pots of each soil were divided into 16 groups of 4 pots each, which were randomized in their arrangement on the benches. The cuttings were planted on August 18, and the experiment was discontinued on November 17, 1942.

In addition to the 2 soils used in these experiments, muck soil was included in one series of another experiment. Composited lots of cuttings, prepared as described previously, were germinated at 22°–23° C. and 35°–36° C. in the sandy and silty clay loam soils, and at 35°–36° C. in the muck soil. The 50 plants for each treatment were divided into groups of 10, which were randomized in their arrangement on the benches. The experiment was begun on January 7 and terminated on March 17, 1944.

Germination at 35°–36° C. extended over a period of from 4 to 17 days from the date of planting, with approximately 75 per cent of the plants emerging within 5 days, and 98 per cent within 10 days. At 22°–23° C., germination extended over a period of from 8 to 59 days, with approximately 30 per cent of the plants emerging within 10 days and 76 per cent within 15 days.

The results of the 3 experiments are summarized in table 2. In each, the percentage of plants developing chlorotic streak symptoms was greater in the silty clay than in the sandy soil, and in the last experiment was greater in the muck. While the differences were not significant, either in the individual experiments or when the data were subjected to the Chi-square test, the results of other experiments which are discussed later sug-

gest the possibility that the consistently though only slightly greater incidence of the disease in the clay soil may have been related to its higher nitrogen content.[3] Even assuming that the differences in nitrogen content of the soils were great enough to affect symptom expression of the disease, however, it is probable that in the field the differences in drainage and moisture content generally associated with the 2 soils would have a greater influence.

The rate at which the plants germinated had no measurable effect on the development of chlorotic streak, those that germinated rapidly showing symptoms to as great a degree as those that germinated slowly. In addition to determining the average percentage of diseased plants for each treatment, as shown in table 2, the plants were arranged in frequency classes of 5-day intervals according to the number of days required for germination, and the percentage of those showing chlorotic streak symptoms determined for each class. The averages for each frequency class were not significantly different from the general average, thus showing no relation between rate of germination and development of the disease.

TABLE 2.—*Incidence of chlorotic streak in sugar-cane plants of the variety C.P. 29/320 grown from infected seed cane in sandy loam, silty clay loam, and muck soil, maintained at or near optimum moisture content*

Temperature of germination in each experiment	No. of plants in each soil	Plants with chlorotic streak			
		Sandy soil	Clay soil	Muck soil	Diff. Req'd. for P. = 0.05
		Per cent	*Per cent*	*Per cent*	*Per cent*
Experiment 1					
35°–36° C.	125	80.3	86.4	.....	.....
22°–23° C.	115	83.2	87.5	.....	.....
Experiment 2					
Min. 24° C.	64	77.1	79.2	.....	12.0
Experiment 3					
35°–36° C.	50	53.1	65.3	62.0	16.2
22°–23° C.	40	52.5	57.5	.....	12.1

Influence of Soil on Recovery from Chlorotic Streak

During the course of these studies, it was observed that chlorotic-streak-infected plants of C.P. 29/320 growing in the greenhouse in muck soil recovered from the disease to a greater extent than those growing in sandy or clay soils. Since this variety rather commonly shows recovery from the disease, experiments were conducted to determine whether the soils would have a similar effect with other varieties in which there ordinarily is less recovery.

Ten infected plants each of the C.P. numbers 29/103, 29/320, 34/139,

[3] According to unpublished analyses by Nelson McKaig, Jr., and L. A. Hurst, the nitrogen content of a typical sample of the Sharkey soil was 0.211 per cent, and of the Yazoo soil, 0.085 per cent. An unpublished analysis by R. L. Holmes gave 0.66 per cent nitrogen for the muck soil.

and 36/85, five growing in Sharkey silty clay soil and five in muck soil, were selected when 6 weeks old for uniformity of size and severity of chlorotic streak symptoms, and transferred from 6-inch pots to 4-gallon cans of the same soil where they remained for 10½ months without the addition of fertilizer. The stalks were then segmented into single-bud cuttings, which were planted in 4-inch pots of sandy loam soil. The percentages of plants in each group that developed chlorotic streak symptoms were: C.P. 29/103—53 per cent (from muck) and 91 per cent (from clay); C.P. 29/320—21 per cent (from muck) and 97 per cent (from clay); C.P. 34/139—85 per cent (from muck) and 87 per cent (from clay); C.P. 36/85—92 per cent (from muck) and 86 per cent (from clay).

Presumably the greater recovery from chlorotic streak that occurred in 2 varieties grown in muck soil was related to higher nitrogen content of that soil, the influence of which is discussed later.

NUTRITION OF THE SUGAR-CANE PLANT

According to Martin (10), the development of chlorotic streak is closely associated with the nutrition of the sugar-cane plant. He found that the leaf symptoms disappeared when diseased plants were taken from the field and transferred to a complete nutrient solution. He also mentioned the observations of field men that the disease was more prevalent and severe in fields of low potash content. Bell (5, 6), on the other hand, found no indication that the disease was associated with plant food deficiencies, although he stated that unbalanced nutrition might accentuate it. He later (7) reported no curative or preventive effects by the addition of Li, Na, Hg, Cu, B, Zn, Mn, Ba, As, Pb, or Fe to the soil.

Some experiments had indicated a relationship between nitrogen nutrition and the incidence of chlorotic streak, and since this is the principal fertilizer element applied to sugar cane in Louisiana a study of its effect on the disease was undertaken.

Nitrogen

The effect of nitrogen on symptom expression of chlorotic streak was first observed when the number of plants with the characteristic leaf streaks increased from 3 to 10 fold among 4- to 6-week-old plants of C.P. 29/320 grown from infected cuttings that had been fertilized with a solution of $NaNO_3$. Experiments were then conducted to study the effect of the nitrogen nutrition of the sugar-cane plant on the development of the disease.

One hundred plants of C.P. 29/320 growing in 4-inch pots of sandy loam soil were divided into 2 lots after the shoots emerged. Each pot in one lot was fertilized with 100 cc. of a solution of $NaNO_3$ containing 10 g. of the salt per liter, and the remainder were left as controls. Notes were made on the appearance of chlorotic streak symptoms.

Sixteen plants of C.P. 29/320 growing in 6-inch pots of sandy loam soil received the equivalent of the $NaNO_3$ in the preceding experiment, but in 5 weekly applications, and an equal number was left as controls.

The results of the 2 experiments are in table 3. Additions of $NaNO_3$ were followed by marked increases in the percentage of plants showing the typical leaf symptoms of chlorotic streak. This effect became evident more slowly when the fertilizer was added in 5 applications, than when all of the nitrogen was added at one application. At the end of the experiments, when the control plants were also fertilized, they developed symptoms to practically the same extent as the originally fertilized ones, showing that the virus was present but did not become evident until the application of nitrogen caused symptoms to develop.

In other experiments it was found that $(NH_4)_2SO_4$, cottonseed meal, and filter press cake[4] as sources of nitrogen also caused an increase in the number of plants developing foliage symptoms, but the effect became evident more slowly than with $NaNO_3$.

TABLE 3.—*Comparative effect on chlorotic streak symptoms of adding equivalent amounts of $NaNO_3$ in 1 application and in 5 applications*

Days after fertilization	Plants with chlorotic streak symptoms			
	N applied once		Five applications of N	
	Fert.	Check	Fert.	Check
No.	*Per cent*	*Per cent*	*Per cent*	*Per cent*
7	20.0	8.0	6.7	12.5
14	46.0	34.0	18.7	18.7
21	60.0	48.0	56.2	25.2
48	83.0	78.0[a]	56.2	50.0[b]

[a] The check plants were fertilized 14 days prior to this reading.
[b] The check plants were fertilized 8 days prior to this reading.

The effect of nitrogen fertilization on symptom expression in plants that had already developed leaf streaks was also studied. Twenty-five 6-week-old plants of C.P. 29/320 and 10 of C.P. 28/19 with leaf streaks, growing in sandy loam soil in 6-inch pots, were fertilized and an equal number left unfertilized as controls. Another lot of 48 plants of C.P. 29/320 was fertilized, and an equal number left as controls. Notes were made on the occurrence of chlorotic streak symptoms on each leaf of all of the plants before fertilization, and again 13 days later. The results are in table 4.

The fertilization resulted in marked increases not only in the number of plants with additional streaked leaves, but also in the percentage of leaves with streaks. The new symptoms appeared more commonly on the leaves unfolded at the time of fertilization than in those unfolding subsequently.

These results raised the question as to whether the intensification of leaf symptoms following nitrogen fertilization was the result of an increase of the virus in the plant, or an accentuation of foliage symptoms resulting from movement of the virus into the leaves in response to the stimulated growth.

[4] Filter press cake is a by-product of the fabrication of sugar from sugar cane. Chemically it is a highly variable product, depending on the season, variety of cane, maturity, and fabrication methods. An average sample from Louisiana factories was estimated by Rands (13) to contain 2 per cent nitrogen.

Spencer (14) found a definite relationship between the concentration of tobacco-mosaic virus and the amounts of nitrogen supplied to plants, and (15) a marked reduction in the biological activity of the virus in plants grown in a nitrogen-deficient sand culture. It seemed desirable, therefore, to study the effect on chlorotic-streak development in sugar-cane plants of providing them with a continuously high level of nitrogen throughout their growth period, as compared with a low level. Knowledge of the influence of nitrogen fertilization on the development of the disease is of practical importance because of the custom in Louisiana of giving heavy nitrogen fertilization to plots of sugar cane being grown especially for seed cane.

TABLE 4.—*Effect of fertilization with $NaNO_3$ on intensification of chlorotic streak symptoms in plants of C.P. 28/19 and 29/320 already showing leaf streaks*

Variety	Number of plants		Chlorotic-streaked plants in which additional symptoms appeared after fertilization		Plants on which leaf symptoms first appeared after fertilization	
	Fertilized	Check	Fertilized	Check	Fertilized	Check
			Per cent	*Per cent*	*Per cent*	*Per cent*
C.P. 28/19	10	5	50.0	20.0	36.4	11.1
C.P. 29/320	25	25	76.0	33.3	14.4	3.1
C.P. 29/320	48	48	52.0	20.0	33.7	6.9

TABLE 5.—*Effect of high nitrogen vs. no nitrogen on recovery from chlorotic streak in 3 varieties of sugar cane*

Variety	Leaves with chlorotic streak		Buds with chlorotic streak	
	High N	No N	High N	No N
	Per cent	*Per cent*	*Per cent*	*Per cent*
C.P. 29/103	48	47	34	52
C.P. 34/139	65	87	80	74
C.P. 29/320	38	68	22	47

Plants of C.P. 29/320 from composited lots of single-bud cuttings from infected stalks were grown in the greenhouse in 6-inch pots of Yazoo very fine sandy loam until they were 2 months old and had developed symptoms of chlorotic streak. Twenty-two were then selected for uniformity as to size and number of leaves with streaks, 11 to be fertilized with $NaNO_3$ and 11 to remain unfertilized. At this time and at intervals of 2 weeks thereafter each plant in the fertilized series was given 200 cc. of a solution containing 10 g. of $NaNO_3$ per liter. When they were 3 months old they were transplanted to 4-gallon galvanized cans of the same soil. A record was kept of leaf symptoms during the 8-month course of the experiment, when the stalks, which then had from 2 to 13 lateral buds, were segmented into single-bud cuttings and planted in the greenhouse.

In a repetition of the experiment, the varieties C.P. 29/103 and 34/139,

which ordinarily show less recovery than the 29/320, were used, with 5 plants in each of the fertilized and control series. The results of the experiments are in table 5.

The high level of nitrogen caused a marked reduction in the number of infected buds of C.P. 29/103 and 29/320. Similar results were obtained with these varieties in a field experiment, in which chlorotic-streak-infected stools of C.P. 29/320 receiving 300 lb. per acre of nitrogen as $NaNO_3$ showed apparently complete recovery, and C.P. 29/103 a significant degree of recovery in comparison with stools receiving the usual plantation rate of 40 lb. of nitrogen per acre. However, with 3 other varieties in the field test, there was no recovery.

Nitrogen and Potassium in Sand Culture

The influence of nitrogen and potassium on development of the disease was studied in sand culture. Plants of C.P. 29/320 were grown in white sea sand with the addition of a complete nutrient solution, until they were 6 to 8 inches tall, and were showing symptoms of chlorotic streak. They were then transferred to 3-gallon crocks of sand, where one series of 5 plants received a complete nutrient[5] containing 165 p.p.m. nitrogen and 208 p.p.m. potassium; a second series, 825 p.p.m. nitrogen and 208 p.p.m. potassium; and a third series, 165 p.p.m. nitrogen and 20.8 p.p.m. postassium. Changes of nutrient were made at weekly intervals, with the leachate poured back daily after making up losses from evaporation with distilled water. At the end of 5 months the stalks were harvested and the buds planted in sandy loam soil.

As in previous experiments, the high level of nitrogen caused a marked reduction in the percentage of infected buds, 35 per cent of those from this series producing diseased plants compared with 88 per cent with the lower level. The low level of potassium caused a marked increase in the occurrence of leaf streaks, approximately twice as many leaves becoming streaked in this series as in that with the normal level, and more than twice as many buds producing diseased plants. Symptoms did not disappear, however, from any of the plants grown in the complete nutrient with all elements at the normal level.

DISCUSSION

Field observations that excessive soil moisture accentuates both the incidence and severity of chlorotic streak were confirmed experimentally in the field and greenhouse. A similar response to excessive soil moisture, especially when accompanied by deficient fertilization, was reported by Jones (8) for carnation yellows, and by Pryor (12) for big vein of lettuce. Presumably, the deficiency of oxygen in waterlogged soils and possibly also the development of toxic compounds not only render the sugar-cane plant more

[5] The solution contained the following salts in the partial volume molecular concentrations indicated: $Ca(NO_3)_2 \cdot 4H_2O$, 0.0058; KH_2PO_4, 0.0005; $MgSO_4$, 0.001; KCl, 0.0025; with the addition of other elements in p.p.m., Fe, 5.0; Mn, 0.25; B, 0.5; Al, 0.5; Cu, 0.25; Zn, 0.25.

susceptible to infection, but favor the development of the virus in the plant after infection takes place. From the standpoint of controlling the disease, it is apparent that improvement of drainage and avoiding the planting of susceptible varieties in poorly drained soils are important.

The level of nitrogen available to the sugar-cane plant had a marked influence on the development of chlorotic streak. A dosage to young plants was followed, with all varieties studied, by the appearance of leaf symptoms earlier and in a higher percentage of the plants as compared with those receiving no nitrogen. Likewise, single high-nitrogen applications to older plants, particularly if they had begun to show indications of nitrogen deficiency, resulted in the appearance of leaf symptoms in many plants previously symptomless, as well as intensification of symptoms in those already streaked. Continuing the heavy nitrogen applications at intervals throughout the life of the plant, on the other hand, eventually caused a decrease in the percentage of infected buds of 2 varieties studied in the greenhouse, and apparently complete recovery in one of them in a field trial. With other varieties, however, there was no significant degree of recovery.

The level of nitrogen required to induce recovery was much higher than would ordinarily be applied to sugar cane in the field, even to that being grown for seed cane. It is doubtful, therefore, whether the amounts of nitrogen commonly used in plantation practice would have any material effect on the prevalence or severity of the disease.

One possible explanation of the recovery that occurred is that in the plants receiving the continuous high level of nitrogen the virus did not multiply in proportion to the rate of growth of the plants. The increase in symptoms resulting from the single large dosage of nitrogen may have been the result of the movement of the virus into the leaves in response to the stimulated growth. Then, in those plants that were supplied with additional nitrogen and continued to grow rapidly, the virus did not increase proportionately so that the ultimate effect was a dilution of the concentration present, resulting in a decrease in the percentage of infected leaves and buds, or in those with sufficient concentration of virus for the characteristic symptoms to become manifest.

While marked deficiencies of a critical element accentuated the severity of chlorotic streak, the affected plants did not necessarily recover from the disease when the deficiencies were corrected. This did not occur in these experiments even with C.P. 29/320, a variety in which there often is considerable recovery from the disease. Judging from the literature and from the results of experiments reported in this paper, the occurrence of the disease is apparently not associated specifically with the deficiency of any particular element but may be accentuated by poor growth conditions resulting from several causes.

SUMMARY

Chlorotic streak developed to a greater degree in both the field and greenhouse in sugar-cane plants grown from infected seed cuttings in poorly

drained soil than in comparable soil with good drainage, and secondary spread of the disease was greater in plants grown from healthy seed cuttings in the field under conditions of poor drainage as compared with good drainage.

In the greenhouse, there was no significant difference in incidence of the disease among plants grown from infected seed cuttings in Sharkey silty clay and Yazoo very fine sandy loam soils.

Applications of nitrogenous fertilizer to young plants grown from infected cuttings increased the number developing leaf symptoms. Large dosages of nitrogen at frequent intervals during the life of the plants caused a high degree of recovery in two varieties in the field and greenhouse, but had no significant effect on other varieties studied.

Recovery in the absence of added nitrogen was greater in plants of two varieties grown in muck soil than in clay soil, which was attributed to the higher nitrogen content of the former soil.

DIVISION OF SUGAR PLANT INVESTIGATIONS,
U. S. DEPARTMENT OF AGRICULTURE,
HOUMA, LOUISIANA.

LITERATURE CITED

1. ABBOTT, E. V. A progress report on the study of chlorotic streak of sugar cane in Louisiana. Sugar Bull. **18**(19): 3–5. 1940.
2. ———— and J. W. INGRAM. Transmission of chlorotic streak of sugar cane by the leaf hopper *Dracculacephala portola*. Phytopath. **32**: 99–100. 1942.
3. BELL, ARTHUR F. 34th Ann. Rept. Queensland Bur. Sugar Exp. Stations, pp. 50–69. 1934.
4. ————. 35th Ann. Rept. Queensland Bur. Sugar Exp. Stations, pp. 39–48. 1935.
5. ————. 37th Ann. Rept. Queensland Bur. Sugar Exp. Stations, pp. 34–41. 1937.
6. ————. 38th Ann. Rept. Queensland Bur. Sugar Exp. Stations, pp. 41–50. 1938.
7. ————. 42nd Ann. Rept. Queensland Bur. Sugar Exp. Stations, pp. 11–13. 1942.
8. JONES, LEON K. Mosaic, streak, and yellows of carnation. Phytopath. **35**: 37–46. 1945.
9. MARTIN, J. P. Chlorotic streak disease of sugar cane. Proc. 5th Cong. Internat. Soc. Sugar Cane Techn., pp 823–829. 1935.
10. ————. Rept. of the 56th Ann. meeting of the Hawaiian Sugar Planters Assn., p. 30. 1937.
11. PRICE, W. C. Virus concentration in relation to acquired immunity from tobacco ring spot. Phytopath. **26**: 503–529. 1936.
12. PRYOR, DEAN E. The big vein disease of lettuce in relation to soil moisture. Jour. Agr. Res. [U.S.] **68**: 1–9. 1944.
13. RANDS, R. D. Filter press cake reduces damage from root diseases in black lands. Sugar Bull. **11**(1): 2–4. 1933.
14. SPENCER, E. L. The effect of host nutrition on concentration of tobacco-mosaic virus. Plant Physiol. **14**: 769–782. 1939.
15. ————. Inhibition of increase and activity of tobacco-mosaic virus under nitrogen-deficient conditions. Plant Physiol. **16**: 227–239. 1941.

CERCOSPORA LEAF SPOT OF BROAD BEAN IN CHINA

T. F. Yu [1]

(Accepted for publication November 27, 1946)

INTRODUCTION

In the broad-bean growing regions of southeastern China, the crop is attacked by a leaf-spotting fungus commonly referred to as *Cercospora fabae* Fautrey. The disease is rather common, but generally is of little economic importance except in the low, poorly drained rice land where bean is rotated with rice. In this case, leaf spot first appears on the dense lower leaves of the plant in early spring. Until March or April, the infected lower leaves, especially during wet weather, either decay or drop and the normal development of the plant is suppressed. Infected plants usually are less prolific than healthy ones.

REVIEW OF LITERATURE

Cercospora fabae Fautrey was first described by Fautrey in 1890 on broad bean collected at Clamercy, Côte-d'Or (2). It has since been reported as occurring in China, Bohemia, France (9), Japan (8), England (5), Cyprus (3), and Italy (6).

Apparently very little work has been done on *Cercospora* leaf spot of broad bean. Woodward (12), in 1932, described in detail the symptoms of the disease, the morphology of the causal fungus, and the results of inoculations. As far as the writer is aware, that is the only paper that reports a comprehensive investigation of this disease.

SYMPTOMS

In the field in China, the disease appears primarily on leaves, only rarely on leaf petioles and stems, and never on flowers and pods.

The first symptom is a chocolate-colored spot about 1 mm. in diameter on the lower leaves. At the end of spring season, spots are seen on the upper leaves. Under favorable conditions, spots enlarge rapidly. The center of a spot becomes light gray with a broad, slightly raised, deep red margin. The lesions are usually zonate. The number of spots on a single leaflet varies from 1 to 20, usually 1 to 4. Spots are oblong, round or occasionally irregular, from 1 to 14 mm. in diameter, the average falling between 5 and 7 mm. These spots may occur on any part of the leaf surface, including tips and margin, and they are not limited by veins.

The appearance and size of the spots vary with climatic conditions. In dry seasons, the spot remains small with depressed center and raised, deep red ridge. The larger spots have distinct margins. In a wet climate, the black central area of the spot extends rapidly and the margin becomes less and less distinct (Fig. 2, B). Numerous conidia are then produced on the

[1] Formerly Professor of Plant Pathology, The University of Nanking, Nanking, China.

center of the spot (Fig. 2, A), which then appears silver gray. Frequently the dead tissue falls out, leaving a shot-hole effect on the leaf.

If several spots are on the same leaf blade, the tissue between them may collapse to form a large dead area. Sometimes, the veins on which spots are located become discolored and the leaf tissue beyond the vein has a tendency to wilt and soon turn black. Large, irregular, dead areas formed by coalescence of the spots may cover a large portion of the leaf surface. When the spots occur on the tip or margin of a leaf the dead tissue soon falls out. The various symptoms of the disease on leaves are shown in figure 1.

FIG. 1. Spots produced by *Cercospora fabae* Fautrey on the leaves of *Vicia faba* L. in the field.

Only in rare cases, does the fungus produce lesions on leaf petiole, stipule, and stem. The lesions on leaf petiole and stem are spindle-shape, or oblong, with gray center and deep red margin, almost indistinguishable from the young lesions produced by *Ascochyta fabae* Speg. During wet seasons, black lesions on these plant parts are not uncommon. Sporulation of the fungus has not been observed on them.

THE CASUAL FUNGUS

Two species of *Cercospora* have been reported on broad bean. One is *C. fabae* described by Fautrey (2) and the other is *C. zonata* described by Winter (11). They presumably differ from each other in size and septation of conidia and in color of conidiophore. These characters are, however,

by no means constant. Welles (10) pointed out that conidia of some species of *Cercospora* may vary in size and septation under different environments. Woodward (12) examined conidia collected from the field in England. Their dimensions were 40–126 × 4–6 μ with an average of 75 × 4.7 μ. The number of septa varied from 3 to 7, the average number being seven. He also showed that conidia from plants grown in a cool greenhouse averaged 93 × 4.5 μ. Their range was 44–171 × 3.5–5.5 μ. The number of septa varied from 1 to 14, the average being seven. Because size and septation of conidia are variable characteristics of the species of *Cercospora*, Woodward believes that *C. fabae* Fautrey and *C. zonata* Wint. may be identical. The writer also found that conidia of *C. fabae* Fautr. vary in size and septation under different environments. The fungus responsible for the leaf-spot disease on broad bean in China therefore is identified as *C. fabae* Fautr.

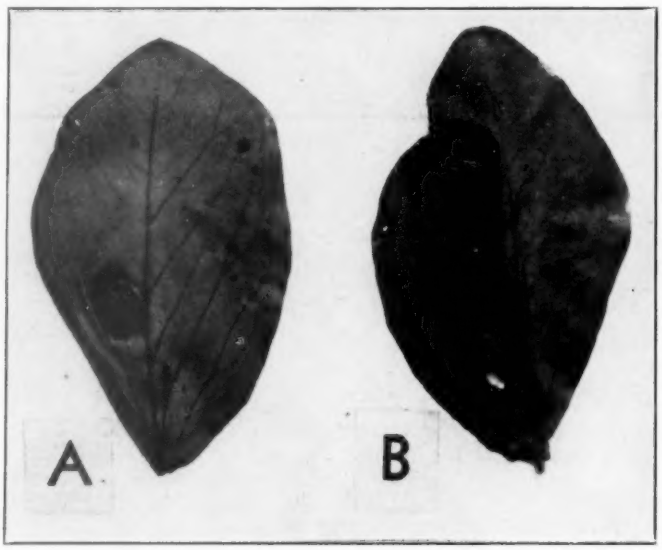

FIG. 2. Cercospora spots on leaves under moist conditions. A. Spot with sporulation and indistinct margin. B. Spot becoming soft and black, the margin of the spot becoming less distinct.

The conidiophores of the present fungus emerge in clusters of 1 to 12, mostly 3 to 6, from the leaf surface. They are brown, 0 to 2 septate, usually simple, and 15.8–99.4 × 4.5–6.0 μ. Conidia, borne terminally, are elongate, tapering above, straight or slightly curved, obclavate, and colorless. Conidia collected from the field measured 29.2–102.8 × 2.8–4.6 μ with an average of 56 × 3.8 μ. The number of septa varies from 2 to 9, the average being seven. The size of 312 conidia, collected from beans grown in a damp rice field, was 34.4–131.7 × 3.2–5.0 μ with an average of 64 × 4.2 μ. The number of septa varied from 1 to 15, the average being nine.

INOCULATION EXPERIMENTS

Pathogenicity of the fungus has been determined by inoculation ex-

periments in the greenhouse. Young and half-grown broad bean plants were inoculated with a water suspension of conidia obtained from a diseased plant grown in the greenhouse. In the present experiments, tiny, water-soaked, red spots appeared 2 to 3 days after inoculation. It took about 10 to 15 days to produce the typical zonate spots characteristic of field infections. Numerous experiments of this sort showed that conidia of the fungus can infect the noninjured leaves of broad bean. It is of interest that Woodward (12) was unable to infect noninjured leaves in his inoculation experiments.

Inoculation of the leaf petiole and stems with suspensions of conidia in the greenhouse occasionally resulted in tiny red spots. The results of these experiments, however, are inconsistent. Apparently, under normal conditions, the fungus does not infect these plant parts.

HOST RANGE

Numerous inoculations were made to determine the host range of the fungus. Leaves of plants to be inoculated were washed with mercuric chloride (1–1000) and rinsed with sterile water. Spore suspension was then sprayed on the upper surface of the leaf. After inoculation, the plants were kept in a moist chamber 48 hr. and then set on greenhouse benches. The fungus was not able to infect the following species of plants: *Pisum sativum* L., *P. sativum* var. *arvense* Poir., *Lens esculenta* Moench, *Vicia sativa* L., *V. cracca* L., *V. villosa* Roth., *Lathyrus odoratus* L., *Phaseolus vulgaris* L., *Vigna sinensis* Endl., *Dolichos lablab* L., *Glycine max* Merr., *Medicago sativa* L., *Melilotus alba* Desr., *Trifolium repens* L., and *T. pratense* L.

CULTURAL CHARACTERS

The fungus has been grown on most of the common culture media. Potato-dextrose agar seemed to be most favorable for its growth. In Petri dishes, the fungus was first white and gradually became light gray. As growth proceeded, the coloring became dark gray to black. The center of the colony was usually slightly raised and at the margin there might be one or two zones present. Sometimes, irregular dark gray or deep olivaceous green patches were on the surface of the colony. In old cultures, the mycelium became dense and compact. Sporulation of the fungus in artificial culture media, by means of the methods suggested by Nagel (4) and Diachun and Valleau (1), could not be induced.

TEMPERATURE RELATIONS

The relation of temperature to fungus growth was judged by the increase in diameter of fungus colonies on agar plates. Each 90-mm. Petri dish contained about 15 cc. of one per cent potato-dextrose agar. Single spores were isolated from the fungus, and small bits of mycelial growth of approximately equal size from young, vigorous colonies were used to plant test plates. Quadruplicate plates were placed in constant-temperature chambers

held at 5°, 10°, 16°, 25°, 27°, 31°, and 33° C. The average diameter of colony was determined at 24-hr. intervals.

Based on the radial growth of the fungus during 6 days at different temperatures, the optimum was about 25° C., with a maximum slightly over 31° C. and a minimum about 5° C. Cultures grew when kept at 5° C. for a long time.

OVERWINTERING

In May, 1928, severely diseased leaves were buried one inch beneath the soil surface, between wire screens. In January, 1929, the decayed leaves with adhering soil were dug out and laid over a large wooden box of soil in which broad-bean seeds had been sown. These boxes were covered by a moistened cloth tent for two days and were set in a cool greenhouse. Sufficient water was added to keep them moist. On February 15, the first symptom of the disease appeared on the lower leaves. Typical *Cercospora* spots were on most of the leaves at the end of the month. A quantity of the decayed leaves with adhering soil was brought into the laboratory. It was soaked in 200 ml. sterile water and the mixture was shaken vigorously for a few minutes. After standing for about 20 min., the supernatant water was centrifuged. Numerous fresh conidia were thus collected. When sprayed on healthy broad-bean seedlings, the conidia caused infection. Microscopic examination of the decayed leaves revealed that either conidiophore clusters or stromatic mycelium of the fungus remained alive throughout the winter. Conidiophores might produce fresh conidia for the primary infection in the early spring when conditions were favorable. When the experiment was repeated in 1933, the result was the same.

In other experiments, diseased leaves were collected in May, 1934, and dried. They were pulverized and kept in cool places. In winter, they were laid about $\frac{1}{4}$-inch thick over the surface of soil in which broad-bean seedlings were growing. The typical disease developed.

These experiments show that the fungus overwinters in diseased leaves.

SUMMARY

A leaf spot of broad bean caused by *Cercospora fabae* Fautr. is very prevalent in southeastern China. The disease is serious in wet seasons in the lower rice land where beans are rotated with rice. In this case, the lower leaves decay and drop in early spring and thus check the normal development of the plant. In the well-drained rice fields and on the uplands the disease is of little economic importance.

Spots, primarily on leaves, are oblong, round, or irregular, mostly 5 to 7 mm. in diam., gray at center, chocolate to red along the margin, and zonate. The diseased tissue may fall out, leaving a shot-hole. Under moist conditions, the infected lower leaves of the plant may either fall or decay.

Inoculations in the greenhouse with suspensions of the conidia show that they may infect noninjured leaves of broad bean. The fungus is confined to

Vicia faba L. and is unable to infect 14 species and one variety of leguminous plants tested.

The fungus overwinters on diseased leaves as conidiophores or stromatic mycelium.

THE INSTITUTE OF AGRICULTURAL RESEARCH,
TSING HUA UNIVERSITY, CHINA.

LITERATURE CITED

1. DIACHUN, STEPHEN, and W. D. VALLEAU. Conidial production in culture by *Cercospora nicotianae*. Phytopath. 31: 97–98. 1941.
2. FAUTREY, F. in C. ROUMEGUERE. "Fungi exsiccati precipue Gallici. Centurie LVI°", No. 5588. Rev. Myc. 13: 13. 1891.
3. NATTRASS, R. M. Annual report of the mycologist for the year 1935. Rep. Dir. Agric. Cyprus 1935: 57–64. 1936.
4. NAGEL, CLATUS M. Conidial production in species of *Cercospora* in pure culture. Phytopath. 24: 1101–1110. 1934.
5. PETHYBRIDGE, G. H. Fungus and allied diseases of crops, 1925, 1926, and 1927. Gt. Britain Min. Agr. and Fish., Misc. Publ. 70. 1929.
6. PETRI, L. Rassegna dei casi fitopatologici osservati vel 1927. Boll. R. Staz. Pat. Veg. 8: 1–50. 1928.
7. RADEMACHER, B. Erfahrungen uber die wichtigsten Krankheiten der Ackerbohne und Bekampfung. Deutsche Landw.-Presse 61: (21) 253–254, (22) 275–276, (23) 296. 1934. Abstr. in Rev. Appl. Myc. 13: 670. 1934.
8. SHIRAI, M. and K. HARA. A list of Japanese fungi hitherto known. 2nd Ed., Tokyo, Japan. 1917.
9. STEVENSON, J. A. Foreign plant diseases. U. S. Dept. Agr. 198 pp. 1926.
10. WELLES, COLIN G. Observations on taxonomic factors used in the genus *Cercospora*. Science 59: 216–218. 1924.
11. WINTER, G. Contributiones ad floram mycologicam lusitanicam. Ser. 5. Bol. Soc. Broter II, 1883: 49. 1884.
12. WOODARD, R. C. *Cercospora fabae* Fautrey, on field beans. Trans. Brit. Myc. Soc. 17: 195–202. 1932.

HELMINTHOSPORIUM ROSTRATUM ON CORN, SORGHUM, AND PEARL MILLET[1]

GEORGE Y. YOUNG, C. L. LEFEBVRE, AND A. G. JOHNSON[2]

(Accepted for publication December 3, 1946)

In 1927 and again in 1928, Bunting[3, 4] reported a species of *Helminthosporium* on corn (Maize) that was considered very close to *H. rostratum* Drechs. His figures show the characteristic form of spores, including the rostrate tips and prominently protruding hila, but there is no indication or

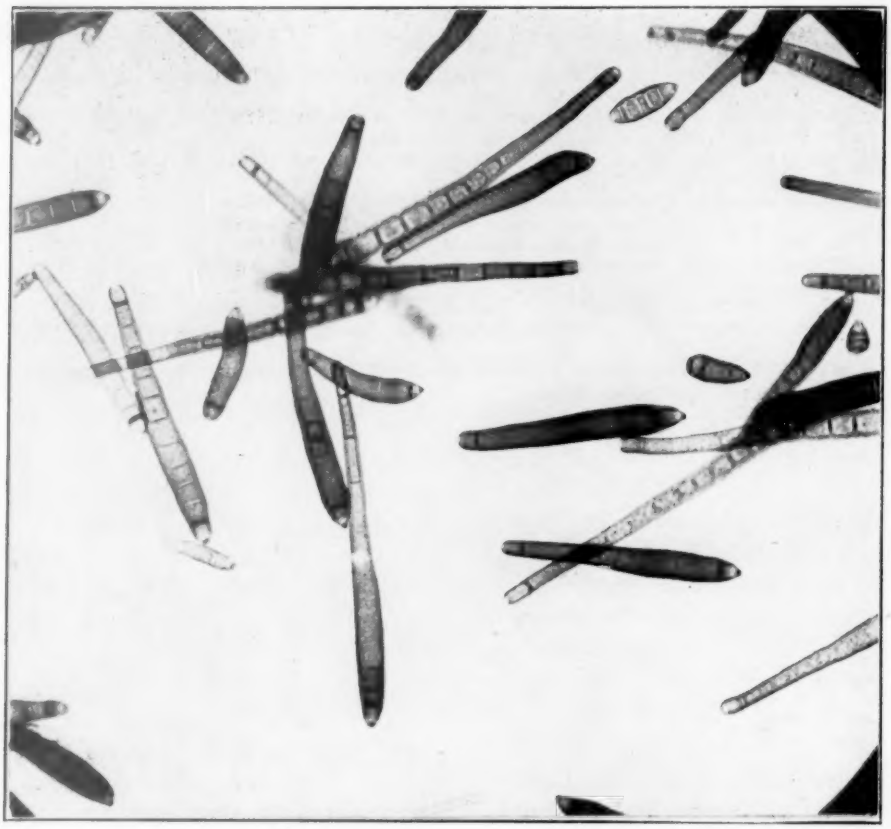

FIG. 1. Conidia of *Helminthosporium rostratum* isolated from Pearl millet collected at Tifton, Ga., 1944. (×360.)

[1] Cooperative investigations of the Divisions of Cereal Crops and Diseases and Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Mississippi Agricultural Experiment Station. Contribution Paper No. 122, New Series, Department of Plant Pathology, Mississippi Agricultural Experiment Station.

[2] The assistance of Helen S. Sherwin in connection with various phases of the work here reported is gratefully acknowledged.

[3] Bunting, R. H. Local cereal diseases in the records of the Mycological Division. Dept. Agr. Gold Coast Yearbook 1926, Bul. No. 7: 25–27. 1927.

[4] Bunting, R. H. Black auricle disease of maize. *In* Fungi affecting graminaceous plants of the Gold Coast. Dept. Agr. Gold Coast, Bul. 10: 19–20. 1928.

description of the dark, thickened terminal septa, so characteristic of this species. These may have been overlooked, however, or their importance as a diagnostic character underestimated. Dade[5] lists the fungus reported by Bunting as "*H.* (?) *rostratum* Drechsl." and cites both of Bunting's reports without comment. In 1941, Lefebvre and Johnson[6] listed *H. rostratum* Drechs. on *Zea mays* L. collected by Lefebvre at Tifton, Ga., in 1940.

In the summer of 1944, a fungus that was clearly *Helminthosporium rostratum* Drechs. was isolated from leaf spots on corn (*Zea mays* L.) from State College, Miss. (Young, Cult. No. 2311), and Tifton, Ga. (Lefebvre, Cult. No. 1847); on Honey sorghum (*Sorghum vulgare* Pers., Cult. No.

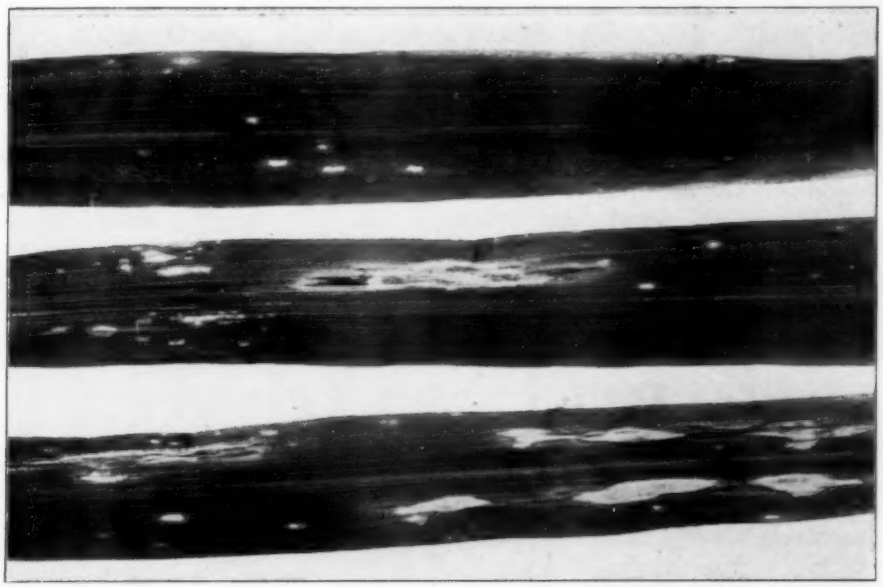

FIG. 2. Lesions on leaves of corn (Inbred Fla. 22) following artificial inoculation with *Helminthosporium rostratum* in the greenhouse at Beltsville, Md., January 1945.

1615), Spur feterita (*S. vulgare*, Cult. No. 1613), Common and Sweet Sudan grass (*S. vulgare* var. *sudanense* (Piper) Hitchs., Cult. No. 1679) from Gainesville, Fla.; and on Pearl millet (*Pennisetum glaucum* (L.) R. Br., Cult. No. 1657) from Tifton, Ga.

While there were slight differences in the spores from the various hosts, the essential characters agreed with those given by Drechsler[7] for the species as originally described from *Eragrostis ciliansis* (All.) Link (*E. major* Host), including elliptical and rostrate tipped spores, the latter with dark, protruding hila, and dark, thickened, distal and basal septa (Fig. 1).

There is a considerable variation in the lesions on the leaves of the various

[5] Dade, H. A. A revised list of Gold Coast fungi and plant diseases. Kew Roy. Bot. Gard. Bul. Misc. Inform. No. 6 (1940): 205–247. 1940.

[6] Lefebvre, C. L., and H. W. Johnson. Collections of fungi, bacteria, and nematodes on grasses. U. S. Dept. Agr., Plant Dis. Rptr. 25: 556–579. 1941.

[7] Drechsler, Charles. Some graminicolous species of *Helminthosporium*: I. Jour. Agr. Res. [U.S.] 24: 722–724. 1923.

host plants. In general, the lesions are small, 1–2 × 2–5 mm., and commonly limited laterally by the leaf veins. Lesions may coalesce, however, to form larger, necrotic areas, which extend across the veins. The necrotic centers of all the lesions, particularly on corn, bleach to straw color, usually with slight browning at the edges and some yellowing at the ends.

On sorghums and sorghum relatives there is more or less purpling except on Leoti sorgo, and Tift and Sweet Sudan grass. On Pearl millet, the lesions at first are rather dark brown and later tend to become light brown, particularly on the older leaves.

ARTIFICIAL INOCULATIONS

In 1945, corn in Mississippi, and at Beltsville, Md., was inoculated with isolates No. 2311 and No. 1847. Both isolates infected corn (Fig. 2) and the fungus was re-isolated from the lesions.

TABLE 1.—*Disease ratings on plants of corn, sorghum and sorghum relatives, and Pearl millet following inoculation in the greenhouse at Beltsville, Md., with cultures of Helminthosporium rostratum from various sources, 1946*

Plants inoculated	Disease ratings following 2 series of inoculations with culture No.											
	2311		1847		1615		1613		1679		1657	
Corn	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
Fla. 4	2	6	1	5	4	7	5	5	2	5	4	7
Fla. 6e–23	2	2	1	4	2	6	3	5	1	6	3	5
Fla. 21e–2	2	7	4	5	5	6	6	5	5	5	6	8
Fla. 22	1	6	1	5	2	6	3	6	1	5	3	7
Yellow Paymaster	5	5	2	5	2	5	2	6	4	4	5	6
Sorghum etc.												
Honey	1	1	1	1	2	2	1	2	1	2	2	3
Leoti sorgo	0	1	0	1	1	1	0	1	0	1	1	1
Spur feterita	1	2	0	2	1	3	1	4	1	2	1	3
Red x	1	1	1	1	1	2	1	1	1	2	2	2
Acc. No. 5	2	2	1	2	2	3	2	2	2	3	3	3
Common Sudan grass	1	2	2	2	2	2	3	2	2	4	2	4
Sweet Sudan grass	1	1	1	1	1	1	1	1	0	1	1	1
Tift Sudan grass	1	1	1	1	1	1	2	1	0	1	2	1
Johnson grass	2	2	1	1	1	3	3	2	1	2	4	3
Pearl millet												
Commercial	0	6	0	3	0	8	0	7	0	8	0	7
Sel. 794	1	6	0	1	0	5	0	7	0	6	0	6

In January, 1946, single-spore cultures from the six sources were used in two series of cross-inoculations at the Plant Industry Station, Beltsville, Md.

The cultures, grown on carrot decoction with dextrose added, were homogenized in a Waring Blendor for two minutes and atomized on the plants, after which the plants were left in a moist chamber for 48 hours. Following this the plants were placed in a greenhouse at about 80° F. and kept well watered.

Plants in series A were inoculated January 28, 1946, when fairly young, that is, from 8 to 10 inches tall. The disease ratings were made February 1. For series B, older leaves of the same plants were reinoculated with the same

cultures, respectively, the latter part of March and disease ratings were made April 1. This procedure was possible as there had been no spread of infection to the later leaves.

The disease ratings were made on the basis of severity of infection on practically all leaves from 0, no infection, to 10, very abundant infection, and various gradations between. Ratings below 3 indicate high resistance.

The plants inoculated and the disease ratings in both series A and B are given in table 1. The most severe infections were obtained on corn and Pearl millet; none of the sorghums or sorghum relatives tested were very susceptible. Leoti sorgo was the most resistant. Sweet and Tift Sudan grass, both of which originated as hybrids between Leoti sorgo and Common Sudan grass, likewise were highly resistant. Pearl millet was susceptible only in the older stage (series B). Usually corn was more susceptible in the older stage (series B) than in the younger stage (series A).

While there were some indications of differences in the pathogenicity of the cultures from the different sources, this phase needs additional study before definite conclusions are justified.

SUMMARY

Helminthosporium rostratum Drechs. has been isolated from leaf spots on corn from Mississippi, and Georgia; on sorghum and sorghum relatives from Florida, and on Pearl millet from Georgia.

Six cultures from the above sources were pathogenic on these various hosts in cross-inoculation experiments.

PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND,
AND
MISSISSIPPI AGRICULTURAL EXPERIMENT STATION.

THE EFFECT OF SEEDLING DISEASES OF CASTOR BEANS ON THE SUBSEQUENT PLANT DEVELOPMENT AND YIELD

E. C. STEVENSON[1]

(Accepted for publication December 16, 1946)

Seedling diseases in commercial plantings of castor beans in the United States have frequently resulted in very poor stands and probably have been an important cause of the low yields that are reported at times for this crop. The damage to the seedlings varies from cases in which the cotyledons are malformed, spotted, and stunted to those in which the seedling is killed. *Alternaria ricini* (Yoshii) Hansford, species of *Fusarium*, and various

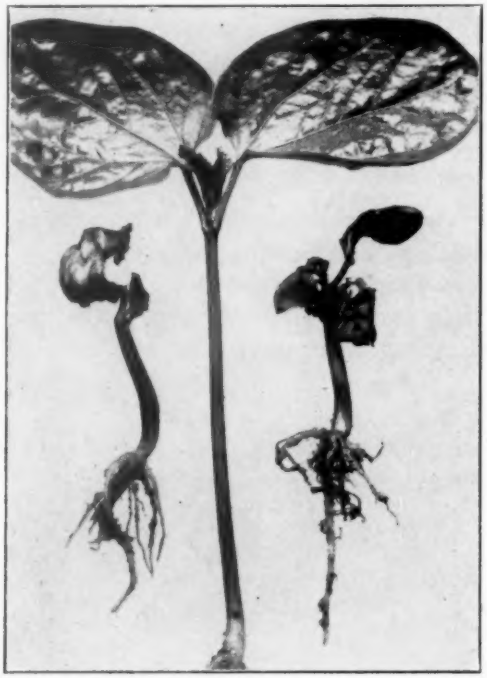

FIG. 1. Seedling blight and die-back of castor bean. The seedling in the center has not been affected while those to the right and left have a common type of injury.

Mucorales have been found by extensive isolation studies to be most frequently associated with these malformed cotyledons and seedling die-back of castor beans at Beltsville, Maryland (Fig. 1).

When only portions of the cotyledons are affected and the growing point is not involved, the seedlings recover and, after the cotyledons have abscissed, the plants apparently develop normally for the remainder of the season. Because such infections of the cotyledons usually occur each year and have

[1] Associate Pathologist, Division of Tobacco, Medicinal, and Special Crops, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration.

been very widespread in some plantings, a study was made during the seasons of 1943 and 1944 to determine the effect of this injury on the subsequent development of the affected plants that survived.

MATERIALS AND METHODS

Seed of the varieties Conner, Kentucky 38, and Doughty 11 were used in the two-year study. The experimental design was a split-plot randomized-block of four replications with varieties occupying the main plots. Each sub-plot contained four rows of ten hills each; only the two center rows being used as data rows. Twelve to 14 seeds were planted per hill and each hill was thinned to one plant before the first true leaves were well formed. The thinning was done in such a manner that two classes of plants made up the sub-plots. These were the "control" class, made up of plants selected at the time of thinning because they were apparently disease-free, and, the

TABLE 1.—*The effect of seedling diseases of the castor-bean plant on seedling survival and the yield of seed per plant in 1943 and 1944 at Beltsville, Maryland*

Comparisons	Average number of plants that survived per plot of 20 plants		Average yield of seed per plant	
	1943	1944	1943	1944
			Grams	*Grams*
Class (all varieties)				
Injured	14.9	19.2	155.93	113.40
Controls	18.6*	19.8	158.76	136.08*
Variety (both classes)				
Conner	17.5	19.7	167.27	127.58
Kentucky 38	16.9	19.0	170.10	119.07
Doughty 11	16.0	19.8	136.08	124.74

* Significantly greater than the corresponding figure for the injured class at the 5 per cent level.

"injured" class containing plants that were selected as being diseased at the time of thinning. Data were taken on the following: (1) number of plants that survived per plot, (2) number of racemes per plant, (3) number of racemes harvested, (4) yield per raceme, (5) yield per plant, (6) yield per plot, (7) hulling percentage,[2] and (8) test weight per bushel. By applying the F test to the error mean squares for each item for the two years it was found that the data were in sufficient agreement to permit a combined analysis for the two years except in the cases of items (1) and (5) where separate analyses were made for each year of the test.

RESULTS AND DISCUSSION

Data on the number of plants that survived per plot and the average yield per plant are presented in table 1. In 1943 there was a statistically significant loss of plants per plot in the injured class because many of the seedlings died subsequent to thinning. Consequently, in thinning the plots

[2] Hulling percentage is that proportion of hulled, sound seed obtained from a given weight of air-dry seed in the hull.

in 1944 only those injured seedlings were left which appeared as if they would survive the disease, thereby making the comparison between the two classes more equable. No significant difference in the number of plants which survived per plot occurred in 1944 when this more selective method of thinning was used. The difference in stand between the two years is reflected in the average yields per plant as shown in the table. In 1943 there was no difference in the average yield per plant between the two classes while in 1944 the individual plant yields in the injured class were significantly less than those of the controls. This apparently was due to the reduced competition between plants in the injured class (since there were fewer plants per plot)

FIG. 2. Reduction in early vigor of castor beans (Conner variety) caused by seedling blight. The plants in the four rows left of center had diseased cotyledons while those to the right of center were not affected.

in 1943 resulting in the production of more seed per plant. In 1944 there was no difference in stand and individual plants in the injured class produced significantly less seed per plant than the controls.

It was obvious immediately after emergence and until mid-season in both years that the plants from diseased seedlings were less vigorous than the controls (Fig. 2). Later this effect was not so pronounced, although the vigor of the former never reached that of the latter.

A summary of the combined analysis for 1943 and 1944 of the other characters studied is presented in table 2. In both years significantly fewer racemes were harvested per plant and per plot from the injured class than from the control class. In the first year this was partially due to the reduced stand of injured plants, but in both years it was caused primarily

by the fact that plants from affected seedlings matured somewhat later and therefore correspondingly fewer racemes from these matured before frost. In the first year the injured plants of the three varieties (Conner, Kentucky 38, and Doughty 11) produced a total yield of seed per plant which on the average was 26.4, 14.4, and 28.7 per cent less, respectively, than that of the control plants. In 1944 the figures for Conner and Kentucky 38 were 35.6 and 18.5 per cent, respectively. In the case of Doughty 11 there was no difference in the yields from the injured and control plants in 1944 but at the time of thinning the number of injured seedlings of this variety was very small, and after thinning the injured plants were therefore much like the controls.

TABLE 2.—*Summary of the effect of seedling diseases of the castor bean on plant development and seed yield. (Each figure is an average of four replications and two years)*

	Racemes harvested per plot	Av. No. racemes harvested per plant	Av. yield of seed per raceme	Seed yield per plot	Calculated yield per acre	Hulling percentage	Weight per bushel
	Number		*Grams*	*Grams*	*lb.*		*lb.*
Class (varieties combined)							
Injured	72.3	4.3	37.42**	2221	870	68	42
Controls	97.0**	5.0*	32.89	2804**	1098**	69	43
Variety (classes combined)							
Conner	61.0	3.3	47.06**	2713	1063	69	42
Kentucky 38	125.6**	7.0**	20.13	2514	985	69	41
Doughty 11	67.2	3.7	38.19**	2311	905	68	45

* Significantly greater than the corresponding figures at the 5 per cent level.
** Significantly greater than the corresponding figures at the 1 per cent level.

The control plants yielded significantly less seed per raceme than the injured plants in both years. This is explained by the fact that the injured plants matured later and the bulk of the yield from this class was obtained from the first or main racemes which usually are much larger than the later racemes in the case of these varieties. In the control plots many of the smaller, later-formed racemes ripened in time to be harvested, thus lowering the average yield per raceme.

The quality of the seed from the two classes of plants was the same since there was no significant difference in hulling percentage and the weight per bushel.

The three varieties did not differ significantly in yield, hulling percentage, and weight per bushel. Kentucky 38 produced significantly more racemes per plot and per plant than either of the other varieties, while Doughty 11 and Conner yielded significantly more seed per raceme than Kentucky 38.

SUMMARY

Seedling infections by various fungi resulting primarily in cotyledonary injury of castor-bean plants had a pronounced effect on the subsequent plant growth and yield even when the affected plants recovered. Affected seedlings that were not killed were retarded more or less in proportion to the extent of the infection. This was indicated by a reduction in height and general vigor as well as by later maturity. The yields of seed in the two years studied were markedly reduced by seedling infections because of the reduced vigor and later maturity. The quality of the seed produced from plants with cotyledonary injury did not seem to be affected as there was no significant reduction in either the hulling percentage or weight per bushel.

PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND.

PHYTOPATHOLOGICAL NOTES

Poria obliqua on Dying Beech.—Poria obliqua (Pers.) Karst. is an important wood-decay organism found principally on species of *Betula,* occasionally on *Ostrya* and *Fagus.* On live hosts it is reported to form only rimose, clinker-like masses of sterile tissue commonly known as sterile conks. Fertile sporophores, apparently, were believed to be produced only on dead trees and only after decay was well developed.[1]

In the vicinity of Tully, New York, August, 1946, a fertile fruit-body of *Poria obliqua* was found on a dying trunk (d.b.h. 5 inches) of *Fagus grandifolia* Ehrh. A part of the lower trunk was alive as evidenced by a living branch, soundness and high moisture content of a portion of the sapwood of the trunk, green cortical cells, and the presence of demonstrable nuclei in the parenchyma of the sound sapwood.

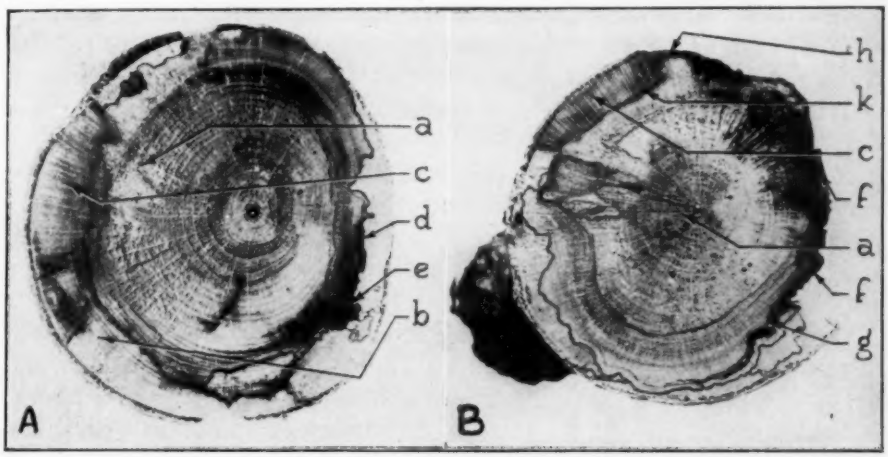

FIG. 1. Cross sections of the trunk of a dying beech on which a fertile sporophore of *Poria obliqua* was formed. A. A section taken directly below the resupinate sporophore. × ⅜. B. A section taken 15 inches above Section A and through the sporophore. × ⅜. a, advanced stage of decay; b, intermediate stage of decay; c, living sapwood; d, black zone line; e, concentrations of hyphae behind the black zone line; f, sporophore; g, mat of mycelium which developed behind the black zone line; h, sporophore tissue adjacent to living sapwood; k, cinnamon brown zone line adjacent to living sapwood.

The portion of the beech stem bearing the fertile fruit-body in close association with living host tissue was cut into sections for study. In cross sections of the trunk (Fig. 1) black zone lines were common to the sapwood and separated advanced decay from earlier stages of decay. In areas where the sapwood was still living the zone lines were cinnamon brown,[2] less distinct, and considerably wider than elsewhere. Microscopic examinations of thin sections through the zone lines disclosed that the lumina of many

[1] Campbell, W. A., and Ross W. Davidson. A Poria as the fruiting stage of the fungus causing the sterile conks on birch. Mycologia **30**: 553–560. 1938.

[2] Ridgway, R. Color standards and color nomenclature. 43 pp. 53 color plates. Washington, D. C. 1912.

cells were filled with swollen brown hyphae, whereas others appeared to contain an amorphous dark-colored substance. A thin mat of mycelium, consisting of brown, heavy-walled hyphae, was present at the edges of the sporophore a few millimeters nearer the heartwood than the black zone line. As this mat of mycelium thickened it apparently loosened a shell of sapwood beneath which the fertile sporophore developed. As the fruit body formed, decayed sapwood and sound sapwood as much as 10 millimeters in thickness were forced off. The sporophore was in intimate contact with the living sapwood.

The presence of the fertile form of *Poria obliqua* on a tree not dead suggests that live trees bearing sterile, rimose conks might advisably be removed from the forest during sanitation cuttings.—ROBERT A. ZABEL, Department of Forest Botany and Pathology, The New York State College of Forestry, Syracuse, New York.

The Observed Frequency of Mature Pycnidia of Septoria gladioli on Gladiolus Corms.—Such references as appear in the literature dealing with the presence of mature pycnidia of *Septoria gladioli* Pass. on the corms of *Gladiolus spp.* emphasize the rare occurrence of this stage. Massey,[1] who first demonstrated the connection of the hard rot of the corms with the leaf spot caused by this pathogen, stated that he failed to find pycnidia on the corms. Papé,[2] who first noted mature pycnidia on hard rot lesions, gives no data on their frequency. Moore[3] writes that he found this fruiting stage "only on three occasions in the last twelve years during which time a very large number of corms affected with Hard Rot have been examined."

The situation came to the writer's attention following the interception of pycnidia with mature conidia on a gladiolus corm in January, 1946, and led to an examination for the mature pycnidial stage in shipments of gladiolus which passed through the Bureau of Entomology and Plant Quarantine inspection house at Hoboken, N. J.

One hundred and fifty shipments examined between January 14 and June 15, 1946, had hard rot lesions. The pycnidial stage bearing mature conidia of *Septoria gladioli* was on 10 of these shipments. This number represents 6.6 per cent of the shipments infected with typical hard rot lesions.

The corms bearing the fruiting pycnidial stage came from Holland, England, Australia, and Canada.

Mature pycnidia are to be sought on corms with an advanced stage of the disease. This may be indicated by the large proportion of the corm covered by the lesions, by confluence of the lesions, and often by the presence of secondary organisms.

[1] Massey, L. M. The hard rot disease of gladiolus. N. Y. (Cornell) Agr. Exp. Sta. Bul. 380: 149–181. 1916.

[2] Pape, H. Die Hartfaule-Krankheit der Gladiolen und ihre Bekämpfung. Die Gartenwelt 29(40): 676–680. 1925.

[3] Moore, W. C. Diseases of bulbs. [Gt. Brit.] Min. Agr. and Fisheries. Bul. 117. 1939.

It is possible that environmental factors provided by packaging for shipment may favor the production of pycnidia. Such factors might include exclusion of light, the accumulation of moisture, and the products of respiration of the corms. But, as some of these shipments arrived in well-ventilated packages, it seems advisable not to exclude the probability that fruiting pycnidia are more common on the corms of gladiolus than has been thought.—DONALD P. LIMBER, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture, Hoboken, New Jersey.

Spotted Wilt of Broad Bean.—During the winter of 1937, attention was called to a serious disease of broad bean, *Vicia faba* L., which was accompanied by symptoms characteristic of the spotted wilt. In the field, a top necrosis of the infected plant was very pronounced. The top portion of the plant shrivelled, blackened, and dried while the lower portion remained green for some time.

The virus was collected from a diseased bean field and was transmitted readily to young tomato plants in the greenhouse. Extracts from young leaves of infected tomato were rubbed on broad-bean seedlings. Typical streak resulted and the percentage of infection was moderate. Young seedlings about 6 inches high were killed within two weeks after inoculation. On old plants, the early symptoms on the leaves were spots, about 1 mm. in diameter, which soon enlarged to form oval or oblong lesions about 1 to 1.5 cm. long with diffuse margin. Later the lesions developed dark-colored zones and blackened. Infected bean leaves first became pale green then grayish black. Yellowish discolored areas were occasionally seen along the necrotic lesions. The leaves curled slightly downward or inward along the tip or margin. Finally, the entire leaf shrivelled. Collapse of the upper leaves resulted in the top necrosis commonly found in the field. Eventually, the lower leaves became involved and the plant was killed by the virus.

On bean stems, the virus produced purplish red streaks of various length, sometimes the entire length of the stem. Streaks were found also on leaf petioles. These streaks gradually blackened and the stems shrivelled and dried. In the field, if plants were infected after pods had formed, the seed was usually small, shrivelled and grayish green with black necrotic spots on the seed coat. The seed seldom germinated to produce normal seedlings.

As a rule, progress of the disease was rapid. The leaves quickly lost their normal greenness, turning to dark gray and collapsing. The whole plant was then killed, or the tops died down except for small lateral shoots. Rarely did an infected plant resume growth after infection.

In the greenhouse the virus was readily transmitted to tomato, tobacco, pepper, and *Zinnia elegans* Jacq. by juice inoculation. It produced the characteristic bronzing of tomato leaves. Small or large necrotic lesions appeared on inoculated tobacco leaves, but the plant usually was not killed.

Brownish black necrotic lesions appeared on inoculated pepper plants and reddish circular lesions developed on inoculated cowpea leaves.

The virus was relatively short-lived in extract. It usually was inactivated after aging for 8 hours in extract at 22° C. The inactivation temperature was approximately 42° C. and the tolerance to dilution was up to 1 : 80,000. Taking all of these properties *in vitro* and the symptoms produced on the various hosts into consideration, the disease of broad bean herein reported is unquestionably caused by spotted wilt virus.

In Chengtu, Szechwan province, China, tomatoes are grown extensively by farmers and spotted wilt has been found in certain tomato fields. The localization of diseased plants in the bean fields suggests transmission of the virus into the field from neighboring tomato plants through the medium of some insect vector.

For many years, extensive surveys of broad-bean diseases have been made and it is the first time the writer has seen broad bean naturally infected with and severely attacked by spotted-wilt virus in the field.—T. F. YU, Department of Plant Pathology, The University of Nanking, Chengtu, China.

A Rough-bark Disease of Pittosporum Tobira.—An apparently undescribed disease of *Pittosporum Tobira* Ait. has been under observation and study in central California at Berkeley and in southern California in West Los Angeles for several years. The disease has also been noted in Albany, near San Jose, in Riverside, and Montebello, and apparently is more prevalent in southern than central California.

The most damaging effect of the disease is necrosis of the outer bark which later loosens and sloughs off (Fig. 1, A). Not infrequently the necrosis extends so deep into the bark that the branch is girdled and killed. Plants so affected have only terminal clusters of a few, small, rolled leaves on the branches. The shrubs are smaller than normal and sometimes slowly die. In addition to the bark symptoms, this disease produces characteristic leaf symptoms (Fig. 1, B). Not infrequently necrotic spots are produced in leaves. Leaf symptoms are of several types. The mildest consists of chlorotic blotches with indefinite margins, yellow on young leaves, tan on old ones; sometimes green islands are included in the areas. Another occasional type has small, angular, yellow areas suggestive of Cercospora leaf spot.[1]

An oak-leaf or water mark pattern occurring particularly along the midrib is common. Ring spots with concentric yellow lines or moiré patterns are less frequent. Similar symptoms were seen on *Pittosporum viridiflorum* Sims at Montebello, and on variegated *P. Tobira* in West Los Angeles, but these plants were not included in this study. No virus-like symptoms have been seen by the writers on *P. undulatum* Vent.

The disease is carried in cuttings from affected plants but is not completely systemic since less than half of such cuttings ever produced symptoms

[1] Plakidas, A. G. Angular leaf spot of Pittosporum. Mycologia 32: 601–608. 1940.

though they were kept under observation for more than two years. It is not carried in seed readily if at all. Fifty-three seedlings from seed of an affected plant and twenty-two from a plant without symptoms were kept under observation for a year or more without developing any symptoms. Among some 200 seedlings on the Los Angeles Campus of the University, no symptoms were seen during the first several years after planting. Although the symptoms seemed obviously to be those of a virus disease, a small-scale inoculation test was made by inarching an affected to a healthy plant of *Pittosporum Tobira*. Both bark and leaf symptoms appeared on part of the inoculated plant within 14 weeks and soon afterward throughout the plant. Cions from a plant of *P. crassifolium* A. Cunn. having virus-like symptoms in leaves but not in bark, and growing near affected *P. Tobira*,

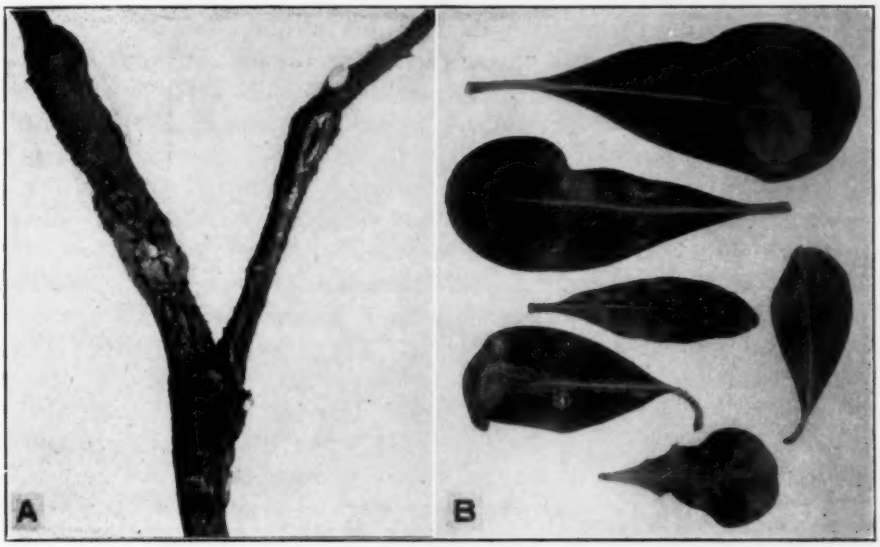

FIG. 1. A. Rough bark on an old plant of *Pittosporum Tobira*. B. Leaf symptoms.

were grafted on healthy *P. Tobira*. After something over five months the leaves of the inoculated *P. Tobira* began to develop symptoms quite similar to those occurring naturally on that species but no bark symptoms had appeared up to seven months from the time of inoculation.

Some indication of natural spread of the disease of *Pittosporum Tobira* was seen in two gardens in Berkeley. In paired plants several years old growing about 20 feet apart, one in each case bore symptoms when first observed. After several years the second plant of each pair slowly developed symptoms. Evidence of natural spread has also been seen at Los Angeles. On the other hand some of the 15-year-old plants near San Jose show symptoms while others in actual contact with them do not. A similar situation prevails in one planting at Berkeley.

It is not yet clear whether this disease of *Pittosporum Tobira* is related

to that which seems to have been transmitted to *P. Tobira* in France in 1887[2] or to that described for *P. daphniphylloides*.[3]

It should be possible to easily control the disease by care in selection of cuttings, by rogueing out affected plants, and by growing plants from seed.—H. EARL THOMAS and KENNETH F. BAKER, Division of Plant Pathology, University of California, Berkeley and Los Angeles.

A Fungicide for Protecting Lily Bulbs from Infection by Colletotrichum lilii.—The use of an eradicant fungicide for controlling bulb-borne infection of Easter Lily (*Lilium longiflorum* Thumb. var. *eximium*) bulbs by the black scale fungus *Colletotrichum lilii* Plakidas has been reported.[1] In more recent work it was found that while the treatment with Puratized N5E destroyed the disease organism in diseased tissue, treated bulbs received little or no protection from reinfection when planted in infested soil.

Since *Colletotrichum lilii* appears to live in the soil for several years, a treatment was sought which would protect the bulbs from infection by soil-borne inoculum. In preliminary greenhouse tests in 1944, infected bulbs, after treatment with Puratized N5X (phenyl mercuri triethanol ammonium lactate), were dusted with Arasan (tetramethyl thiuram disulfide) before being planted in potted, naturally infected soil. When dug about seven months later, all of the nondusted bulbs bore black scale lesions, while only 10 per cent of the Arasan-dusted bulbs were infected (Fig. 1). The disease indices (See footnote b in table 1) were 71 and 4 for nondusted and Arasan-dusted bulbs, respectively.

The use of Arasan as a protectant against soil-borne infection was then studied in field trials in the 1945–46 season in three different tests as follows:

1. A comparison of the Puratized N5E treatment with and without Arasan dust and Arasan dust alone with nontreated diseased bulbs in soil which had produced a severely diseased crop the previous year (1945).

2. Same as 1, except the soil had not been planted to Easter Lilies in 1945, but had produced a severely diseased crop in 1944. Nontreated bulbs were not included in this test, nor was Arasan used alone.

3. Clean disease-free bulbs dusted with Arasan were compared with untreated clean bulbs in soil which had produced severely diseased bulbs in 1945.

In experiments one and two, the Puratized N5E was used at a strength of 1–2000. The bulbs were treated for 48 hours in this solution. The Arasan was applied 24 hours after the bulbs were removed from the Puratized solution, and the bulbs were planted immediately. Where the Arasan was used without the Puratized treatment, the dry bulbs were dusted with Arasan immediately before planting.

[2] Carriere, E. A. Influence du greffon sur le sujet. Revue Horticole 59: 58–59. 1887.
[3] Milbrath, D. G. Probable virus disease of *Pittosporum daphniphylloides*. Monthly Bull. Calif. Dept. Agr. 29: 158–159. 1940.

[1] LeBeau, F. J. The eradicant action of a fungicide on Colletotrichum lilii in lily bulbs. Phytopath. 36: 391–393. 1946.

Experiments 1 and 2 were run in Plaquemines Parish and experiment 3 was located in Terrebonne Parish.

The results obtained are summarized in table 1.

Photographs of representative bulbs from each of the first three treatments of experiment 1 are shown in figure 1. It is evident that the Puratized N5E alone did not protect the bulbs from reinfection, while Arasan alone was not effective since it did not possess sufficient eradicant action to destroy infection already in the bulbs. The combined treatment, however, was highly effective. When clean bulbs were used, the Arasan treatment

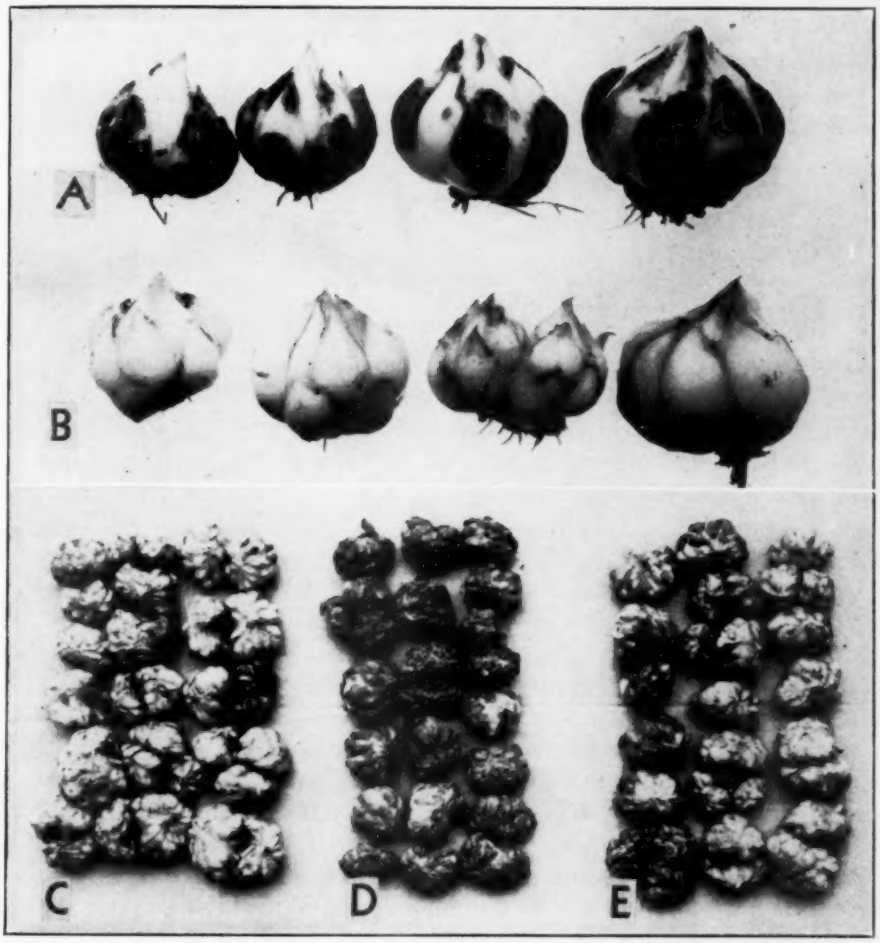

FIG. 1. Effects of Arasan and Puratized N5E on the development of black scale on lily bulbs planted in infested soil. (A) Infected bulbs treated with Puratized N5E only and grown in infested potted soil for 7½ months. (B) Same as A except Arasan was dusted on the bulbs after the Puratized N5E treatment. (C) Diseased bulbs receiving both treatments before being planted in infested soil in the field, and harvested 11 months later. (D) Same as C but with no treatment before planting. (E) Same as C except that only the Puratized N5E treatment was used.

TABLE 1.—*The effects of different treatments on the development of black scale on Easter Lily bulbs grown in infected soil*

Experiment No.	Treatment	No. of bulbs[a]	No. of bulbs in disease classes				Disease index[b]
			Clean	Mild	Mod.	Severe	
1	None	100	0	0	7	93	97.7
1	Puratized N5E	232	76	111	49	68	48.9
1	Puratized N5E followed by Arasan	250	222	24	4	0	4.3
1	Arasan	100	0	24	35	41	72.3
2	Puratized N5E	900	545	135	124	106	26.0
2	Puratized N5E followed by Arasan	672	610	32	20	10	5.1
3	None	134	46	40	20	28	41.0
3	Arasan	150	131	16	3	0	4.9

[a] Results are from 3–4 replications with the exception of the nontreated lot and the Arasan treatment in experiment 1. Infected bulbs were used for experiments 1 and 2; disease-free bulbs were used for experiment 3.

[b] Clean, mild, moderately and severely diseased bulbs were given numerical values of 0, 33.3, 66.6, 100, respectively. The disease index for any given treatment was obtained by summing the products of the numbers of bulbs in each class and the numerical values of the classes and dividing by the total number of bulbs in the treatment.

alone sufficed to prevent the development of black scale in infected soil.—F. J. LEBEAU, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

The Effects of Carborundum in Inoculating Bean Plants with Bacteria.—In experiments reported elsewhere,[1] the carborundum technique for inoculation with viruses[2] yielded excellent results in inoculations with the wound parasite *Pseudomonas ribicola* Bohn and Maloit on *Ribes aureum* Pursh. To study the effect of carborundum in inoculations with bacteria that attack host leaves through stomata, the susceptible Black Valentine variety of kidney bean, *Phaseolus vulgaris* L., was inoculated with *Pseudomonas phaseolicola* Dowson and *Xanthomonas phaseoli* (E. F. Sm.) Dowson. Pertinent literature on the latter diseases was reviewed by Harter and Zaumeyer.[3]

Fully expanded primary leaves and first trifoliate leaflets were dusted with 300-mesh carborundum and rubbed with a cotton pad wet with water or with a suspension of one or the other of the parasitic bacteria. Paired primary leaves or first trifoliate leaflets on the same plants were similarly rubbed but without the application of carborundum. The inoculations were made on seedlings in individual pots in the greenhouse without the use of a moist chamber. An individual plant was used for only one type of inoculation.

The water checks did not develop any infection, but those dusted had

[1] Bohn, G. W., and J. C. Maloit. Inoculation experiments with *Pseudomonas ribicola*. Phytopath. **35**: 1008–1016. 1945.

[2] Rawlings, T. E., and C. M. Tompkins. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopath. **26**: 578–587. 1936.

[3] Harter, L. L., and W. J. Zaumeyer. A monographic study of bean diseases and methods for their control. U. S. Dept. Agr. Tech. Bul. 868. 1944.

small brown flecks that resulted from mechanical injury. In the inoculations with *Pseudomonas phaseolicola*, 1 of 30 dusted leaves or leaflets and 3 of 45 nondusted ones were free from infection; spots were considerably more numerous on the dusted organs (Fig. 1). In the *Xanthomonas phaseoli* series, 2 of 5 nondusted leaves escaped infection while all of the dusted leaves developed severe infection. Similar results were obtained with plants inoculated in the morning, in the afternoon, and at night.

The effects of adding carborundum to suspensions used to spray Black Valentine bean plants in the field at Cheyenne, Wyoming, were studied in 1943. In the control block alternating rows were sprayed with tap water

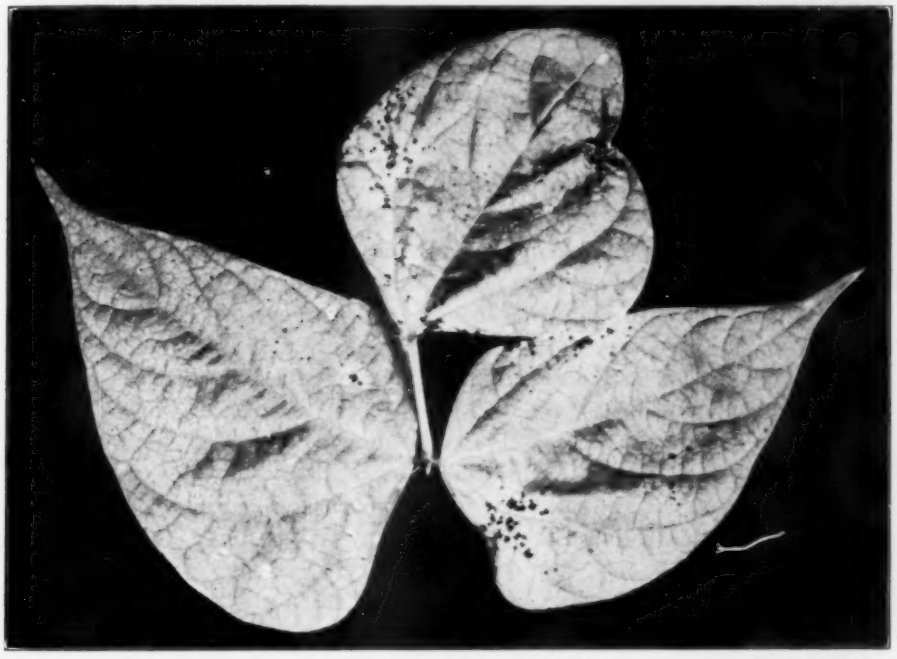

FIG. 1. Leaflets of the kidney bean, not dusted at left and dusted at center and right, rubbed with a cotton pad wet with a suspension of *Pseudomonas phaseolicola*.

or with a suspension of 300-mesh carborundum in tap water (20 g. in a liter). In the test block, odd-numbered rows were sprayed with a $\frac{1}{6}$ dilution in tap water of beef-extract-dextrose-broth cultures of *Pseudomonas phaseolicola;* even-numbered rows were sprayed with the same suspension with carborundum added. After spraying between 6 and 7 p.m. on July 22, a mist was maintained over the plants for 2 hours with an overhead-spray irrigation system.

After 20 days, numerous bacterial spots had developed on all plants sprayed with the bacterial suspensions. No apparent difference in numbers of spots resulted from the use of bacterial suspensions containing carborundum and those lacking it. No injury was seen on leaves of the controls sprayed with water either with or without the carborundum. Apparently,

carborundum applied in this manner caused few additional infection courts for the bacteria. It is obvious that carborundum is unnecessary to obtain abundant infections with stoma-invading parasites if experimental conditions favor abundant ingress through stomata.

These experiments suggest that carborundum powder may be useful in inoculations with stoma-invading parasites under conditions that do not favor ingress of the parasite through stomata but is of little value under conditions that do favor such ingress. These experiments, together with those on bacterial spot of currant cited above, suggest that carborundum may be found most useful with wound-parasites and with hosts that have hard, glabrous, thickly cutinized leaves.—G. W. BOHN, formerly Associate Plant Pathologist, Cheyenne Horticultural Field Station, and J. C. MALOIT, formerly Agent, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

Efficacy of Certain Soil Fumigants and Fertilizers against Crown Rot in Annual Larkspur Caused by Sclerotium rolfsii.[1]—A commercial flower grower in the Rio Grande Valley of Texas has experienced extensive losses from crown rot caused by *Sclerotium rolfsii* Sacc. in his annual larkspur (*Delphinium ajacis* L.) for several years. Treatments, in August, 1944, of parts of one field with several materials, including chlorpicrin and Iscobrome No. 2, showed promising control of *S. rolfsii*. In December of 1945 fertilizers and soil fumigants were applied in plots in the part of this field that was known to be heavily infested with sclerotia of *S. rolfsii*. Soil fumigants were included to determine whether the 1944 observations could be substantiated, and the fertilizers were included to determine whether control of *S. rolfsii* similar to that obtained by Leach and Davey in California[2] could be obtained in the Rio Grande Valley.

There were 12 treatments (Table 1) applied to plots 9 feet wide by 18 feet long, and each treatment was randomized in each of 6 blocks. The soil in this field was very low in organic matter, had a pH of 5.6 when tested in 1944 (but probably was more alkaline in 1946), and consisted of a very fine sand and clay. Water used for irrigation was alkaline. The soil fumigants were applied December 12 and 13, 1945, chlorpicrin with a Larvajector, and the other fumigants with a Mack Anti-Weed gun. The soil was very dry at the time of application (1.9–2.2 per cent moisture) and the soil temperature was 73–74° F. at the 6-inch depth. No water seal was applied. The Uramon and ammonium nitrate in treatments 10 and 11 were broadcast uniformly over the plots December 11. A period of cold weather, locally known as a "Norther," prevailed from December 10 to 13, during which time it was cold and cloudy with occasional drizzling rain and with air temperatures of 35–65°. This probably tended to retard volatilization of the gases from the

[1] Thanks are due J. O. Davis, Manager, Weatherford's Farms, Linn, Texas.

[2] Leach, L. D., and A. E. Davey. Reducing southern sclerotium rot of sugar beets with nitrogenous fertilizers. Jour. Agr. Res. [U.S.] **64**: 1–18. 1942.

soil. On January 9, 1946, seeds of larkspur, variety Imperial Los Angeles, were seeded in 3 rows running the length of each plot. The fertilizers in treatments 5 to 9 were divided into 3 equal lots and applied as sidedressings on the following dates: January 30, February 20, and March 20, 1946. Complete flower-production records could not be obtained from these plots, but the healthy larkspur plants in the middle row of each plot were counted April 5, 1946, when plants were just beginning to flower, and large numbers had been killed by or were dying because of *Sclerotium rolfsii*. The data are in table 1.

TABLE 1.—*Number of healthy larkspur plants at flowering following certain soil-fumigant and nitrogen-fertilizer treatments for the control of Sclerotium rolfsii in the Rio Grande Valley of Texas*

Materials	Applications		Rate per acre		Healthy larkspur plants
Soil fumigants	Amt. per injection (cc.)	Spacing of injections (inches)	Fumigant (gal.)	Fumigant (lb.)	Number[c]
1. Chlorpicrin	2.5	12	28.7	402	237
2. Carbon disulphide	20.0	18	102.2	1073	185
3. Iscobrome No. 2[a]	5.0	12	57.5	575	199
4. ETN mixture[b]	10.0	18	51.1	470	170
Nitrogen fertilizers	**Method and time**		**Fertilizer (lb.)**	**N (lb.)**	**Number[c]**
5. Ammonium sulphate	3 sidedressings at monthly intervals		735.0	150	144
6. Ammonium nitrate	do		450.0	150	248
7. Cyanamid	do		712.5	150	155
8. Uramon	do		356.8	150	154
9. do	do		712.5	300	189
10. do	Broadcast before planting		950.0	400	18
11. Ammonium nitrate	do		1200.0	400	19
12. Control, not treated	.....		.....	.....	115

[a] Xylol, 60 per cent; methyl bromide, 15 per cent; and chlorpicrin, 25 per cent.
[b] Ethylene dibromide, 15 per cent; tetrachlorethane, 20 per cent; naphtha thinner, 65 per cent.
[c] Least difference necessary for significance with odds of 19 : 1, 66.7; odds of 99 : 1, 88.5.

All of the materials resulted in an increased number of healthy plants, with the exception of Uramon (a urea compound) and ammonium nitrate broadcast at the rate of 400 pounds per acre before planting. These two treatments were injurious to the growth of the plants. Of the fumigants, chlorpicrin gave excellent control of *Sclerotium rolfsii* as evidenced by the number of healthy plants. Iscobrome No. 2 and carbon disulphide gave fair control. Three sidedressings with ammonium nitrate totaling 150 lb. nitrogen per acre also gave excellent results but, with the exception of Uramon at 300 pounds of nitrogen per acre, none of the other nitrogen-carrying fertilizers was more effective statistically than no treatment.

Leach and Davey reported equally effective control of *Sclerotium rolfsii* in sugar beets in California with equivalent amounts of nitrogen from ammonium sulphate, anhydrous ammonia, calcium nitrate, and Cyanamid. In laboratory tests, however, low concentrations of ammonia in aqueous solution were toxic to mycelium and sclerotia of *S. rolfsii*, ammonium sulphate in alkaline solutions were mildly toxic to mycelium, and calcium nitrate was nontoxic. They were unable to explain this inconsistency satisfactorily. In the Rio Grande Valley tests ammonium sulphate did not control the disease whereas equivalent amounts of nitrogen from ammonium nitrate resulted in good control. Possibly the nitrate ion is more effective than the ammonium ion in the Rio Grande Valley soil, or the ammonium nitrate may have remained available over a longer period of time, resulting in greater inhibition of growth of the *S. rolfsii*. There is also the possibility that ammonium nitrate causes a greater change in the soil microflora, antagonistic to *S. rolfsii*, in Texas than do the other fertilizers.—W. D. MCCLELLAN, Division of Fruit and Vegetable Crops and Diseases, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Maryland.